

EFFECT OF DROUGHT AND SUBSEQUENT RECOVERY ON ENDANGERED
FOUNTAIN DARTER HABITAT IN COMAL SPRINGS, TEXAS.

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EFFECT OF DROUGHT AND SUBSEQUENT RECOVERY ON ENDANGERED
FOUNTAIN DARTER HABITAT IN COMAL SPRINGS, TEXAS

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ABSTRACT

EFFECT OF DROUGHT AND SUBSEQUENT RECOVERY ON ENDANGERED FOUNTAIN DARTER HABITAT IN COMAL SPRINGS, TEXAS

by

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The Edwards Aquifer of central Texas contains several aquatic endemic species dependent on the groundwater discharged from numerous springs, including those that comprise the Comal and San Marcos rivers. One of these endemics, the federally-endangered fountain darter *Etheostoma fonticola* is distributed in only these two rivers of the Guadalupe River drainage. Spring endemic fishes like the fountain darter can be more susceptible to extirpation and extinction events due to their stenothermal requirements and limited distribution. In 2007, central Texas endured drought conditions that spanned two years, culminating with the drought reaching D4-Exceptional status in the summer of 2009. This study evaluated the effect of drought,

recovery, and a subsequent flood on fountain darter habitat, diet, invasive gill parasite levels, and associated fish and macroinvertebrate community structure and composition. Within all three periods, fountain darters were collected in greatest densities in *Riccia fluitans* habitat. Fountain darters were collected in highest densities during the drought with the fewest fountain darters collected during the post-flood period. Available stands of *Riccia* during the drought were diminishing, whereas they were sparse if present post-flood. During the recovery period, fountain darter captures decreased, corresponding with the reemergence of *Riccia* stands within the system. Fish community structure displayed highest species richness, abundance, and evenness during the drought period, though diversity was highest during the recovery period.

Macroinvertebrate community structure had the highest abundance and greatest diversity during the drought, while species richness was greatest during the recovery period. High scores on these indices during the drought period are likely a result of these organisms being concentrated to what available habitat was present in the system. Fish community composition varied between drought and post-flood, and recovery and post-flood periods, indicating the post-flood period had a larger role in characterizing the assemblage than the other two periods. Macroinvertebrate community composition varied among all three periods. This is likely due to the seasonal nature of macroinvertebrates, whom have varied life histories including timing of egg deposition and emergence times. Average prey items per digestive tract were highest during the drought, likely due to prey items being concentrated in habitats. The fewest prey items per digestive tract occurred during the post-flood period, possibly resulting from a lack of vegetation available to inhabit. Prey composition varied among all three periods, also

likely influenced by seasonal trends and the generalist feeding behavior exhibited by the fountain darter. Gill parasite densities were greatest during the drought period, however, these densities did not exhibit any influence from the measured abiotic variables.

CHAPTER 1

EFFECT OF DROUGHT AND SUBSEQUENT RECOVERY ON ENDANGERED FOUNTAIN DARTER HABITAT IN COMAL SPRINGS, TEXAS

INTRODUCTION

Stream ecosystems associated with the karst Edwards Aquifer of central Texas are threatened due to increased urbanization and resulting habitat degradation, eutrophication, and decreased spring flows as a result of minimal recharge and over-withdrawal of groundwater. Several endemic organisms are dependent on the groundwater discharged from the many springs that emanate from the Edwards Aquifer, including the large Comal and San Marcos springs. One such endemic is the federally-endangered fountain darter *Etheostoma fonticola*. In 2007, central Texas endured drought conditions that spanned two years, culminating with the drought reaching D4-Exceptional status in the summer of 2009. We evaluated fountain darter habitat, including macroinvertebrate and fish community structure, during the drought and subsequent recovery, to better understand mechanisms involved in species persistence during disturbance periods.

The natural flow regime paradigm postulates that the structure and function of a riverine ecosystem, and the adaptations of its riparian and aquatic species, are dictated by the pattern of temporal variation in river flow (Poff *et al.* 1997). Five components of the

natural flow regime regulate ecological processes: magnitude, frequency, duration, timing, and rate of change of hydraulic conditions. Ecological processes that are applicable to riverine systems include natural disturbance regimes (drought, flood), hydrological processes (sediment transport), nutrient cycles (carbon, nitrogen, phosphorus), and biotic interactions (predation, competition). Rivers and streams are dynamic in nature and understanding ecological processes during both stable periods and disturbance periods allows resource managers to promote sustainability of the resource (Karr and Chu 2000).

Resh *et al.* (1988) defined disturbance as any relatively discrete event in time that disrupts ecosystem, community or population structure, and that changes resources, availability of substratum, or the physical environment. Disturbance periods are further characterized by a frequency, intensity, and severity outside of the normal range (Dekar and Magoulick 2007). Flood and drought are two common disturbance periods, yet there is a paucity of information regarding how organisms adapt in response to drying periods (Lytle and Poff 2004). Drought, in particular, is difficult to define as it lacks a distinct beginning, and often can only be identified after occurring over a period of time. Humphries and Baldwin (2003) propose using 'drought' as a general term for an unpredictable low-flow period, which is unusual in duration, extent, severity or intensity. Additionally, droughts are characterized by a sequential decline in precipitation, runoff, soil moisture, groundwater levels, and stream flow that may ultimately involve the cessation of surface flow (Acuna *et al.* 2005). Furthermore, droughts can be classified as seasonal (predictable and periodic), or supra-seasonal (longer and unpredictable, marked by a lingering decline in precipitation and water availability), whereby stream biota exhibit low to moderate resistance and a variable resilience to supra-seasonal events (Lake 2003).

When evaluated together, resistance and resilience provide an index of ecological stability of stream ecosystems (Miller and Golladay 1996), with stable communities defined by both high resistance and high resilience to change. Drying periods have been recognized as an important feature structuring fish and invertebrate assemblages, which are often dominated by resistant and resilient taxa. The ability of aquatic organisms, including fishes, to rebound from a disturbance period is key to survival. The ability of these organisms to cope with environmental variability differs among species and is based on life history patterns (Schlosser 1985). Survival of stream biota is often tied to refuge availability. The ability of biota to remain in or seek out suitable habitat is facilitated by habitat patches that experience reduced disturbance, and it is the organisms in these patches that may act as a source of colonists when conditions are more favorable (Davey and Kelly 2007). Those fishes that find suitable refuge experience greater survival and reproductive success (Magoulick and Kobza 2003, Love et al. 2008). Suitable refuge offers cover from larger predatory fishes, provides areas for spawning and egg deposition, and is home to heterogeneous invertebrate prey populations that are often critical to fish survival, particularly in larval fishes.

Periods of disturbance can also affect population numbers and composition of fishes. In a study of wetland fishes, Reutz et al. (2005) observed that densities of fishes (bluefin killifish: *Lucania goodei*, least killifish: *Heterandria formosa*, and the golden topminnow: *Fundulus chrysotus*) were at their very lowest after a drying period, and often required years to return to pre-disturbance densities. Another species in this system, the eastern mosquitofish (*Gambusia holbrooki*) had no time dependencies in recovery, and often recolonized soon after the drying period due to high tolerances to poor water quality

conditions. In the case of flood disturbance, Yoon *et al.* (2011) describe how fish assemblages are significantly affected after major flood, finding that post-flood abundances were nearly half pre-flood observations, and that four species (eastern brook lamprey: *Lampetra reissneri*, goldfish: *Carassius auratus*, spindled loach: *Cobitis lutheri*, and torrent catfish: *Liobagrus andersoni*) had completely disappeared after the flood.

Spring endemic fishes are more susceptible to extirpation and extinction events due to their stenothermal requirements and limited distribution. Fishes that have narrow water quality requirements (stenothermal, stenohaline, etc) may be particularly susceptible to extirpation and extinction events. In particular, spring fishes are one group at a particularly high risk due to increased sedimentation, water extraction, habitat destruction and invasion by exotic species. When spring flow volumes decrease, the fishes that favor eurythermal conditions thrive and gain habitat at the expense of the spring endemic fishes. This interaction between stenothermal and eurythermal fishes is exhibited within poeciliids, cyprinids, centrarchids, and percids (Hubbs 2003). Compounding effects of disturbance to spring systems include fish stocking, invasion by exotic species, and habitat disturbance. Individual springs may be affected by multiple types of disturbance, and the combination of human disturbance with the localized distribution of spring species can lead to changes in the spring fauna, including the loss or imperilment of spring specialists (Bergey, Matthews, and Fry 2008). Bain *et al.* (1988) conclude that fish community structure is strongly influenced by habitat composition and stability, where habitat stability is a function of the flow regime.

Drought periods can also affect invertebrate populations, and thus may subsequently dictate where fish communities are established. Fenoglio *et al.* (2007) found that during de-

watering periods in an Italian perennial stream, taxa that utilize aquatic dispersal (Crustacea, Irudinea, and Mollusca) diminished and eventually disappeared in downstream reaches. More motile forms of invertebrates respond in ways that could have deleterious effects on the previously inhabited space. Ephemeroptera and simuliid species have been shown to increase drift in times of flow reduction as a response to decreased velocities and available habitat (James, Dewson, and Death 2007). Havens, East and Beaver (2007) found that cladoceran densities significantly declined with decreased water levels during a planned dewatering and subsequent unforeseen drought in Lake Okeechobee, Florida. These survival strategies leave the previously inhabited area denuded of invertebrates that otherwise constitute a large portion of food for fishes.

Drought also alters biotic aspects of the environment, changing the physical habitat on both longitudinal and lateral scales, often leading to disconnected pools. This disconnectivity results in isolated patches of suitable refuge for stream organisms and alters the biogeochemical makeup of the stream (Dahm *et al.* 2003). Droughts have a major impact on the interface between ground waters and surface waters and influence the metabolic processes that affect the availability of dissolved oxygen, the distribution of aerobic and anaerobic microbial processes, and concentrations of redox reactive solutes (Dahm *et al.* 2003). Varying levels of turbidity have differing effects on the habitat availabilities in spring systems. Karstic aquifers exhibit turbidity characteristics that are a result of precipitation events versus discharge events (Bouchaou *et al.* 2002). During precipitation events, karstic conduits are subjected to increased discharge rates leading to the destruction of layer limits of clays resulting in the unclogging and releasing of particles which are transported out through the conduits (Bouchaou *et al.* 2002).

There have only been a few studies examining the role of drought on karst spring endemics. Bergey, Matthews, and Fry (2008) evaluated fish assemblage changes in Oklahoma springs over a twenty year interval. Three surveys were conducted on the same 50 springs in 1981, 1982, and 2001, looking to evaluate faunal shifts with respect to flow permanence and habitat alterations. Flow permanence was shown to contribute to higher overall fish abundances, diversity, and species richness. Two species (striped shiner: *Luxilus chrysocephalus* and redbfin darter: *E. whipplei*) were found in the 2001 survey which the authors accounted as a result of increased spring discharge. Habitat alterations contributed to a decline in fish abundances, as game fish (largemouth bass: *Micropterus salmoides* and bluegill: *Lepomis macrochirus*) were introduced to some spring runs that actively preyed on spring endemics, as well as altering habitat previously inhabited by native fishes. Hubbs evaluated the response of several species of *Gambusia* in the Chihuahuan Desert (2003), reporting the necessity of spring flows for the maintenance of spring species, as Pecos gambusia (*G. nobilis*), Big Bend gambusia (*G. gaigei*), and Amistad gambusia (*G. amistadensis*) are restricted to stenothermal portions of springs or spring runs, and are outcompeted by the western mosquitofish (*G. affinis*) in times of decreased flows when the western mosquitofish overlaps with these species. The Amistad gambusia has since gone extinct in the wild as its spring complex has been inundated to form Amistad Reservoir. In a study over disjunct spring fish populations in Oklahoma, Seilheimer and Fisher (2010) found that populations of southern redbelly dace (*Phoxinus erythrogaster*), redbelly chub (*Nocomis asper*), and least darter: (*E. microperca*) are restricted to spring outflows, further stating that the disconnectivity between populations makes them highly susceptible to extirpation should spring flows cease. Although these studies have added valuable insight

as to the response of fishes to drought periods, there is a lack of information on the effect of drought on the ecology of karst spring systems.

During the summer of 2009, central Texas experienced record high temperatures and near record low precipitation resulting in critical low flows from regional springs (Nielson-Gammon and McRoberts 2009). This drying period provided an opportunity to evaluate habitat response to alterations in the flow regime in one of the affected spring systems that includes unique spring-endemic fauna. Comal Springs is located along the Balcones fault line in the Edwards Aquifer and consists of four major springs with associated spring runs, two large springs without spring runs, and three springs below Landa Lake (the impounded headwaters), all of which contribute to form the 4 km long Comal River (Brune 1981). Collectively, Comal Springs forms the largest spring system in Texas with an average discharge of 300 cfs (Gibson, Harden, and Fries 2008). Seven federally threatened or endangered organisms inhabit Comal Springs, including one federally-listed fish, the fountain darter *Etheostoma fonticola*. Examination of the effects of a significant drought on this system would be very beneficial towards understanding the ecological needs of this species and how it responds to corresponding changes in habitat.

The fountain darter is the smallest species of darter (Page and Burr 1979) and was listed as endangered in 1970 (U.S. Office of the Federal Register 35: 16047) due to its limited distribution in two spring-fed rivers in central Texas. At a near constant temperature and pH the Comal Springs system provides a hydrologically stable environment that mitigates most factors that can affect stream systems such as unstable temperatures, varying pH, and the effects of high nutrients. The stability of Comal Springs enables fountain darter populations to persist. Reproduction of the fountain darter is at its optimum within a

temperature range of 19°C-23°C (Schenck and Whiteside 1977), while low turbidity levels of the system allow for efficient foraging as it relies on visual cues for locating and consuming prey, often sitting stationary until its prey comes close enough to consume (Schenck and Whiteside 1977). Most darters are benthic insectivores and it has been suggested by Henry and Grossman (2008) that prey abundance within a microhabitat is a better predictor of habitat use by darters, than are abiotic factors alone. Fountain darters thrive in habitats where vegetation grows near the substrate, such as bryophytes and filamentous algae, (Schenck and Whiteside 1976, Alexander and Phillips, *in press*), and under the usual flow conditions of the Comal River, algal mats are often prevented from extending upwards through the water column, allowing both bryophytes and algae to be available.

Although Comal Springs is fairly stable, a non-native nematode parasite, *Centrocestus formosanus* was introduced to the system in the early 90s, and is now permanently present and may thrive during periods of low flow. This parasite invades and encysts the gill tissue of the fountain darter, causing inflammation and when present in high numbers can affect survival. In a study of the Comal River and neighboring San Marcos River, Mitchell *et al.* (2000) concluded that high levels of cyst accumulation on gill tissue of fountain darters caused higher mortality.

Recreational use is another stress on the Comal River ecosystem. During the summer months, the Comal River is intensely used for swimming, wading, tubing, and fishing. All of these activities contribute to increasing levels of turbidity within the system. Increased turbidity levels can have negative effects on both flora and fauna, impeding vegetation growth and possibly affecting foraging and spawning by fishes. It is also unclear if

increased turbidity levels have any effect on parasite levels. The risk of drought coupled with the invasive parasite and the increased anthropogenic effects to the habitat are compounding recovery efforts for this species.

In addition, there is a precedence of the effect of a significant drought on the fountain darter. During the 1950's, Comal Springs endured a six-year drought that culminated in the cessation of spring flow for six months. This drying period resulted in the extirpation of the fountain darter from the system. It was later reintroduced in 1975-1976 by Southwest Texas State University personnel (Schenck and Whiteside 1976).

The effect of the extreme drought of the 1950s to Comal Springs combined with increasing water extraction from the aquifer, fewer precipitation events and rising temperatures, introduction of an exotic parasite, and the increasing effects of human recreation, it was imperative to evaluate response mechanisms of the fountain darter to disturbance. The data collected here will allow us to better understand ecological responses by fountain darters and will provide insight into possible remediation efforts that would be needed to preserve wild populations in times of stress. This study evaluated habitat use by fountain darters, associated fish and macroinvertebrate community structure and composition, fountain darter diet, and gill parasite levels with respect to drought, recovery, and a subsequent post-flood period by testing the following hypotheses:

- 1) Fountain darter densities will differ among the disturbance periods, more specifically, densities will be highest during the recovery period
- 2) Fish and macroinvertebrate composition, abundance, and richness will differ among disturbance periods with highest richness and abundance during the recovery period

3) Fountain darter diet items will differ among periods, with total prey items available being greatest during the recovery period

4) Parasite infection levels will differ among periods with levels being greatest during times of drought

MATERIALS AND METHODS

Study Area

Comal Springs consists of four major springs with associated spring runs, two large springs without spring runs, and three springs below Landa Lake (the impounded headwaters), all of which contribute to form the 4 km long Comal River (Brune, 1981). The study was conducted in Landa Lake and the Comal River main stem, (Comal County, Texas, USA), monthly for one year (August 2009 – July 2010). Five spring-associated sites (Figure 1) and three vegetation types known as fountain darter habitat within these sites were sampled for presence of *E. fonticola*: filamentous algae (*Rhizoclonium* sp – hereafter referred to as algae), *Riccia fluitans* (hereafter referred to as *Riccia*), and *Ludwigia repens*/*Hygrophila polysperma* (combined here due to similar growth forms, and hereafter referred to as *Ludwigia*).

Vegetation within sites was mapped with a Trimble GeoXT GPS unit (Trimble, Sunnyvale, CA, USA) to ensure sampling consistency. The area of each microhabitat or vegetation type was determined and one-third of this area was sampled to account for differences in area while maintaining consistency. Vegetation type, percent cover, and substrate (silt, sand, gravel, or cobble) was recorded per site.

Water quality variables were recorded at each site within individual microhabitats. Microhabitat depth (m) and instantaneous flow velocity (m/s) was taken with a flow rod and

flow meter (Flo-mate 2000, Hatch Environmental, Loveland, Colorado). Dissolved oxygen (mg/L), pH, conductivity ($\mu\text{S}/\text{cm}$), and temperature ($^{\circ}\text{C}$) were measured with a Hydrolab (Hydrolab, Hatch Environmental, Loveland, Colorado). All probes and multi-meters were calibrated prior to use.

Water samples were taken at each site prior to initiation of any other sampling to analyze for levels of turbidity. Nalgene bottles were rinsed three times with river water before final capture and stored on ice until transport back to the lab for analysis. Levels of turbidity were analyzed with a turbidimeter (DRT-15CE, HF Scientific, Fort Meyers, Florida). Calibration occurred prior to analysis with a primary standard following methods (Standard Methods for the Evaluation of Water and Wastewater 1995).

Etheostoma fonticola Collection

Fountain darters were collected using a fine-mesh (1/16in) 16 x 16 in. D-frame net. Standardized dip net procedure consisted of holding a dip net at arm's length and dipping a distance comparable to one arm's length back to the body (~2ft), moving in an upstream direction between dips. Captured fountain darters were measured for total length (TL-mm) and sexed. A subset ($n = 5$) of total fountain darters captured were randomly selected for diet analysis and placed in a bucket containing a solution of Tricaine methane sulfonate (MS-222; Finquel, Argent Chemical Laboratories, Inc., Redmond, Washington) at a concentration ≥ 250 mg/L. To ensure euthanasia, specimens remained in solution for an additional ten minutes following cessation of opercular movement (Schreck and Moyle 1990). Preservation of specimens was followed by placement in 80% ethyl alcohol (EtOH).

Fish Assemblages

Fish assemblages were quantified via dip net and snorkel survey. All fishes captured via dip net were measured, tallied, and identified to species using Hubbs (2008) and Thomas *et al.* (2007). To account for fishes not captured during dip netting and to observe habitat use by fishes, snorkel surveys were conducted quarterly. Dip net and snorkel surveys were chosen as an alternative to electrofishing and seining to minimize impact to critical habitat and listed species. Snorkeling can provide estimates of abundance similar to electrofishing without the associated injuries, stress, and mortalities (Joyce and Hubert 2003). Surveys began downstream of individual microhabitats and proceeded in a zig-zag pattern in an upstream direction (Lonzarich 2004). All fishes were identified to species and recorded. To minimize observer error, one observer conducted all snorkel surveys.

Macroinvertebrate assemblages

Macroinvertebrate assemblages were assessed to evaluate potential prey items, intermediate host densities, and characterize any flow related shifts. Community makeup of macroinvertebrates were quantified using a Slack sampler (500 μ m mesh) outfitted with a dolphin bucket (500 μ m mesh) at the cod end, and a (30cm x 35cm) quadrat placed directly upstream of net (Moulton *et al.*, 2002). The vegetation patch within quadrat boundaries was agitated for two minutes to release invertebrates downstream into the Slack sampler. Once invertebrates were captured, the dolphin bucket was removed from the sampler, and rinsed with ethyl alcohol into storage jars. All macroinvertebrates collected were preserved in 80% ethanol. All macroinvertebrates were identified to lowest possible taxa using Burch (1982), Thorpe and Covich (1991), and Merritt and Cummins (2008).

Gut Analysis

Diet analysis was performed for evaluation of prey items in the gut. Prior to dissection, fountain darters were sexed and re-measured for total length. Gut content was analyzed from the posterior end of the esophagus to the anterior end of the small intestine (Orr 1989, Bergin 1996). Gut contents were fixed in 10% formalin prior to identification to allow prey tissues to harden, which allowed partially digested prey to stay intact (Murphy and Willis 1996) allowing for easier identification. Dissected stomachs were rinsed with de-ionized water into a petri dish and contents analyzed under a dissecting microscope. Invertebrates found were counted and identified to the lowest taxonomic level using Thorpe and Covich (1991) and Merritt and Cummins (2008). Upon completion of analysis, dissected fish and stomach contents were placed in vials and preserved in 80% ethyl alcohol.

Parasite Sampling

Fountain darters were examined for levels of *Centrocestus formosanus* infestation by inspecting gills for flared opercular flaps. Flared opercles are a result of hypertrophy of the underlying gill tissue in response to infection. To quantify the level of infection, wet mounts of infected gill tissue were examined. Gill arches from the right side were removed and mounted on slides for viewing under a microscope. All cysts were counted per arch, doubled, and used as an estimate of the total number of cysts per fish (Mitchell *et al.* 2000).

Period Characterization

Three periods from the year long study, henceforth referred to as periods, were designated as drought, recovery or post-flood. To standardize periods, lengths were restricted to the minimum tenured period, in this case the drought period in effect lasted for two months

during this study, therefore, all subsequent periods were restricted to this same time frame. The drought period (August – September) averaged 192 cfs and encompassed the time during the initiation of the study, which occurred during an already designated drought, until the return of the seasonal rains. The recovery period (March – April) averaged 359 cfs and was designated as the period when spring discharge returned to a level similar to that of the twenty year average (~306 cfs). The post-flood period (June – July) averaged 502 cfs and refers to the time period after an intense rain event occurred that caused changes in the aquatic landscape. Overall, spring discharge averaged 352 cfs for the duration of this study (Figure 8a).

Data Analysis

Univariate analyses were conducted using program R (v.2.9.2) and (SYSTAT v.12).

Multivariate analyses were performed using Canoco (v.4.5). All data were log transformed prior to analysis to standardize the distribution.

Habitat relationships – Overall site characterization was determined by Principle Component Analysis (PCA) using Canoco for Windows version 4.5. Principle component analysis aims to reduce the dimensionality in a data set that has several interrelated variables, while trying to retain as much of the variation present in the given data set. Thus, it is a data reduction technique that uses principle components to characterize any variation present. In the PCA analysis, the abiotic characteristics of each site including pH, DO, conductivity, velocity, turbidity, and temperature were analyzed. All data were z-transformed prior to analysis. Canonical Correspondence Analysis (CCA) was used to evaluate assemblage habitat associations. Canonical correspondence analysis is a direct gradient analysis that can identify the influence of environmental factors on biotic

communities through examining correlations between two sets of variables. The CCA focused on inter-sample distances using Hill's scaling which allows distances between groups containing nominal data to be interpreted. In order to explore the species-environment relationship, this analysis included all local habitat variables detected from the PCA. The abiotic characteristics of pH, DO, conductivity, turbidity, velocity, depth, and temperature, were included alongside the biotic variables of fish and macroinvertebrate densities, in the analyses respectively. To test the significance ($p < 0.05$) of the variation explained, a Monte Carlo randomization test (1000 permutations) was performed on each CCA model.

Fish and Macroinvertebrate assemblages - Fish densities were determined by total number caught per square meter. Microhabitat used by fishes (as described by depth, velocity, substrate, and percent vegetation cover) were assessed with one factor analysis of variance (ANOVA) for each species by each habitat variable. Fishes were quantitatively compared among sites and independently for each microhabitat category. Analysis of variance was used to evaluate differences in overall fish densities, as well as fountain darter densities, both spatially and temporally. Linear mixed effects analysis was used to evaluate fountain darter microhabitat associations with respect to depth and season. Analysis of variance was used to evaluate differences among periods regarding richness and abundance. Species relative abundance (%), taxa richness, Simpson's diversity, and Pielou's evenness were calculated for each period.

Macroinvertebrate densities were determined by the total caught per square meter. Analysis of variance (ANOVA) was used to evaluate differences in macroinvertebrate densities, both spatially and temporally. ANOVA was also used to evaluate differences

among periods regarding richness and abundance. Species relative abundance (%), taxa richness, Simpson's diversity, and Pielou's evenness were calculated for each period.

Community structure and composition – Fish and macroinvertebrate assemblage structures across periods were characterized by species abundance, species richness, species diversity, and species evenness. Diversity was calculated using the Simpson's Diversity Index (Δ):

$$\Delta = \frac{1}{\sum n_i^2}$$

where n_i is the total number of individuals belonging to i species and N is the total number of individuals. Simpson's diversity index measures the probability that two individuals randomly selected from a sample, will belong to the same species (Horn 1966). Species evenness was calculated using Pielou's (Pielou 1966) Evenness Formula (J'):

$$J' = \frac{1}{H'}$$

where S is the total number of species and H' is the Shannon-Weiner index. Evenness is a measure of the relative abundance of the different species making up the richness of an area.

Bray-Curtis similarity (Bray and Curtis 1957) and Jaccard similarity (Jaccard 1908) indices were calculated for each assemblage. The Bray-Curtis similarity index is an appropriate index for exploring biological community similarities, as it is robust, and the data matrices often have the same units of measure (Clarke and Warwick 2001). This index ranges between zero (completely similar), to one (completely different), and is well suited for abundance data, as it ignores variables that have zeroes for both sets (point absences), and stresses variables with high values because these variables are more likely to be different between sets. The Jaccard index is useful for determining the similarity and diversity of two sets of data. This index ranges from zero (completely different) to 100 (completely similar). It does not take into account negative matches and any similarities are

not influenced by other factors in the analysis and is independent of sample size. These indices were then tested with an analysis of similarities (ANOSIM; Clark and Green 1988) to test for similarities within assemblages among periods. A one-way ANOSIM with period as a factor was then performed to assess hydrologic event effects on assemblages ($\alpha=0.05$; 9,999 permutations). Multidimensional Scaling (MDS) was used to represent dissimilarities between periods in a two dimensional ordinate space.

Diet Analysis – To evaluate the diet of the fountain darter, ANOVA was used to compare the percent composition of the macroinvertebrate groups found in the digestive tracts by site and date. ANOVA was also used to evaluate diet by period (drought, recovery, and post-flood). These analyses were only conducted for the top four taxa found in the diet and environment (however, since we analyzed by period, some taxa overlapped, while others were absent and represented by other taxa): *Hyalella* sp., Cyprididae, Calanidae, *Chironomini*, *Tricorythodes* sp., *Baetis* sp., and *Tanytarsini*. Spearman's rank correlation analysis was then performed on log transformed prey item percentages found in digestive tracts and in the environment. Percent composition and frequency of occurrence in the diet of the fountain darter were determined according to Murphy and Willis (1996).

The linear food selection index (L , Strauss 1979) was used to determine prey selectivity:

$$L = r_i - p_i$$

where r_i and p_i are relative abundances (expressed as proportions) of prey item i in the gut and habitat respectively. Strauss index values range from -1 to +1, with values near -1 representing avoidance, values near +1 representing selectivity, and values near zero representing no selection. Percent of prey items in gut and frequency of occurrence in

environment were calculated for length of study, per period, and for month within each period. ANOVA was used to evaluate differences in diet spatially, temporally, and per period.

Gill parasite analysis – Analysis of variance was used to compare both spatial and temporal levels of infection on gill tissue. A multiple regression was performed to explore abiotic influences contributing to levels of infection. Linear regressions were then performed on any significant results detected in the multiple regression analysis.

RESULTS

Changes in habitat among periods

All three microhabitat types (*Riccia*, *Ludwigia*, and algae) were observed during the drought and recovery period. However, during the drought, algal mats increased in area by growing both laterally across the river bottom, and vertically throughout the water column. When present, this growth blanketed *Riccia* patches, and was observed within *Ludwigia* and other vegetation stands not evaluated in this study.

An October rain event (resulting in a flow of 4290 cfs) flushed the vast majority of algal mats from the system. This rain event uncovered underlying *Riccia* patches (considered quality habitat in this study), and exposed substrate. This marked the beginning of increased overall spring discharge, which averaged 296 cfs for the remainder of October, and saw the return of large *Riccia* patches.

Between the drought and post-flood periods, the Comal Springs system was characterized by spring discharge returning to above the twenty year average (recovery period average - 306 cfs), as well as the reemergence of fountain darter microhabitat types throughout the system. In June following a large rain event, stream discharge increased up

to 7200 cfs (Figure 8a) and scoured the majority of the of the stream bottom leaving the system heavily denuded of vegetation.

Abiotic habitat characteristics (temperature, dissolved oxygen, turbidity, conductivity, depth, current velocity) were compared across periods and some significant differences were observed (Table 1). Temperature ($F_{2,51} = 9.367$, $P < 0.001$), dissolved oxygen ($F_{2,51} = 14,457$, $P < 0.001$), and turbidity ($F_{2,51} = 3.800$, $P < 0.05$) were significantly higher during the drought ($24.2 \text{ }^{\circ}\text{C} \pm 0.3$; $7.65 \text{ mg/L} \pm 0.3$; $7.21 \text{ NTU} \pm 3.1$, mean \pm SE), as opposed to during the recovery ($22.96 \text{ }^{\circ}\text{C} \pm 0.3$; $5.88 \text{ mg/L} \pm 0.2$; $1.01 \text{ NTU} \pm 0.2$), or post-flood ($23.91 \text{ }^{\circ}\text{C} \pm 0.1$; $6.29 \text{ mg/L} \pm 0.2$; $1.15 \text{ NTU} \pm 0.2$). The pH (7.47 ± 0.1) was significantly higher during the post-flood than during the drought (7.23 ± 0.1) and recovery (7.14 ± 0.0) (pH ($F_{2,51} = 4.809$, $P < 0.05$)). Conductivity ($F_{2,51} = 0.583$, $P < 0.562$), depth ($F_{2,51} = 0.137$, $P = 0.872$), and current velocity ($F_{2,51} = 0.236$, $P = 0.236$) were not observed to be significantly different among periods (Table 3).

Principal Component Analysis (PCA) revealed distinct separation of abiotic variables among all five sites (Figure 2). Principal components axis I and II (PC I and PC II) cumulatively accounted for 83% of total variation among sites. Principal components axis I accounted for 64% of the variation and separated sites along a longitudinal gradient. Negative loadings on PC I were temperature (-0.86) and conductivity (-0.44), whereas positive loadings along PCI were turbidity (0.92), pH (0.90), and dissolved oxygen (0.83). Although PC I exhibited a longitudinal gradient from headwaters to downstream, PC II accounted for 19% of the variation and exhibited a separation among microhabitats dictated by favorable conditions. Negative loadings along PC II were temperature (-0.86) which were represented by all shallow algae sites, whereas positive loadings along PC II were

turbidity (0.92), pH (0.90), and current velocity (0.89). Positive PC II loadings characterized the higher current velocity tolerant microhabitats of *Ludwigia* and *Riccia*.

Community structure and composition

Fishes – Overall - There was a total of 5,849 fishes collected from the five sites throughout the year (Appendix A-2). Sixteen taxa were represented over five orders. The order Cyprinodontiformes (79%) was the most abundant, represented by one family and three genera, followed by Perciformes (19%), with three families and eight genera, Cypriniformes (0.8%) with one family and three genera, and Characiformes (0.5%) with one family and one genus. The order Siluriformes was the least abundant (0.03%) with one family and two lone specimens collected within one genus.

The most common species collected was the Western mosquitofish, *Gambusia affinis*, which was present at all five sites (at 70 of the 107 sampling events: 65%). Other commonly observed species with relatively high abundance include the fountain darter (16%), redspotted sunfish (*Lepomis miniatus*) (2%), bluegill (*L. macrochirus*) (1%), and the sailfin molly (*Poecilia latipinna*) (1%). Less commonly observed species include the greenthroat darter (*E. lepidum*) (0.09%), Texas logperch (*Percina carbonaria*) (0.03%), and the yellow bullhead (*Ameiurus natalis*) (0.03%). Total fish collected varied significantly spatially ($F_{8,10251} = 54.200$, $P < 0.001$), with the greatest individuals collected occurring at the main Landa Lake site: 3B ($n = 4,201$), and the least individuals collected occurring at out lowest most site: 5A ($n = 74$). The most individuals collected ($n = 5,037$) by microhabitat type occurred in *Riccia* (74%) (Figure 10a). Total fish collected also varied significantly temporally ($F_{11,10248} = 14.293$, $P < 0.001$), with the most fish collected occurring in August ($n = 2,199$), and the fewest collected in July ($n = 149$). The greatest

total fish collected occurred during the drought period ($n = 3,064$; 69%; Figure 10b). Within the microhabitats, 73% of all fishes were collected in *Riccia*, while 22% were collected in *Ludwigia*, and 4% of all fish collected occurring in algae (Appendix A-3). Sites were coded using site location (1-5) and microhabitat sampled (A = algae, B = bryophyte; and L = *Ludwigia*).

Texas logperch, Texas shiner (*Notropis amabilis*), and the greenthroat darter were associated with higher velocities and higher dissolved oxygen (CCA species biplot; Fig 3). The majority of the centrarchids did not display any strong environmental associations, which is not uncommon as this family of fishes has a high tolerance to a wide range of habitats. One exception was the smallmouth bass (*Micropterus dolomieu*), which was associated with cooler temperatures and increased conductivity, alongside the Mexican tetra (*Astyanax mexicanus*) and the Guadalupe roundnose minnow (*Dionda nigrotaeniata*). More tolerant taxa collected with no strong associations to any environmental variables include both gambusia species found in this system, the large spring gambusia (*G. geiseri*) and the western mosquitofish, the Rio Grande cichlid (*Cichlasoma cyanoguttatum*), largemouth bass, yellow bullhead) and the fountain darter. The mimic shiner (*N. volucellus*), was strongly associated with high turbidity.

Fishes – Focal Periods - There were a total of 3,816 fishes collected among the focal periods (Table 2). The drought period resulted in the most fish captures ($n = 2824$), highest richness (11), and highest Pielou's evenness ($J = 0.48$). The most abundant fish caught was the western mosquitofish (88%), followed by the fountain darter (9%) and the redspotted sunfish (1%). The recovery period included the second highest fish captures ($n = 580$), richness (10), and Pielou's evenness ($J' = 0.47$), but also had the highest Simpson's

diversity score ($\Delta = 0.73$). The most abundant fish during the recovery period were the western mosquitofish (58%), the fountain darter (36%), and the redspotted sunfish (2.8%). The post-flood period had the lowest total fish captures ($n = 412$), richness (5), Simpson's diversity ($\Delta = 0.62$), and Pielou's evenness ($J' = 0.36$). The most abundant fish captured during the post-flood were the western mosquitofish (50%), the fountain darter (44%), and bluegill (3%).

There were a total of 946 fountain darters collected throughout the study. Total fountain darters collected varied significantly spatially ($F_{8,934} = 43.230$, $P < 0.001$) with the greatest number of fountain darters collected occurring at site 3B, in Landa Lake, and the fewest at site 1L, just downstream of Blieders Creek (Figure 13). The fountain darter showed no affinity to a preferred substrate as this system is dominated by cobble/gravel with varying degrees of silt. Overall, 77% of all fountain darters collected occurred in *Riccia*. Total fountain darters collected also varied significantly temporally ($F_{11,931} = 10.318$, $P < 0.001$) with the greatest densities collected during the month of August and the fewest collected in February (Figure 13). Of the 946 captured, 631 (67%) were collected among the three focal periods, with 240 (25%) collected during the drought, 209 (22%) during the recovery, and 182 (19%) during the post-flood ($F_{2,940} = 26.129$, $P < 0.001$).

Of the fountain darters collected, there were significantly more females observed than males ($T(637)$, $P < 0.001$). Thirty-six percent were classified as juvenile, or having a total length less 20mm. Among the three sampled vegetation types (Figure 14), females ($F_{2,387} = 14.689$, $P < 0.001$), males ($F_{2,246} = 6.692$, $P < 0.001$), and total darters ($F_{2,944} = 37.833$, $P < 0.001$), were collected in higher densities in the *Riccia* than in algae or *Ludwigia*. Although juvenile darters were also collected in greater densities in the *Riccia*,

the differences were not significant among the three vegetation types ($F_{2,305} = 0.091$, $P = 0.913$). Fountain darters were also collected on bare substrate following the flood. Areas previously vegetated were denuded by the flood period leaving bare cobble and gravel. Although fountain darters were collected post-flood, densities were far less than pre flood collections.

The first two axes on the fish assemblage CCA (Figure 3a) accounted for 37% of total variation explained by the system as a whole. Of the 37% explained by the model, 26% was explained by canonical axis (CA) I, whereas CA II explained an additional 11% of total variance in the community composition. Canonical axis I represented an environmental gradient from turbidity (0.52), dissolved oxygen (0.22), and pH (0.14) to conductivity (-0.14) and average depth (-0.02). The environmental gradient along CA II displayed positive loadings of pH (0.31), dissolved oxygen (0.22), and average velocity (0.16) to the negative loadings of temperature (-0.11), conductivity (-0.09), and turbidity (-0.02).

The western mosquitofish ($n = 4,595$), largespring gambusia (*G. geiseri*; $n = 27$), Rio Grande cichlid (*Cichlasoma cyanoguttatum*; $n = 26$), and the fountain darter ($n = 946$) plotted out near the origin of the CCA species bi-plot, indicating the presence of these species was less dictated by abiotic variables (Figure 3b). Texas logperch ($n = 2$) and Texas shiner ($n = 24$) were collected in areas characterized by higher pH and dissolved oxygen, whereas the largemouth bass ($n = 14$), Mexican tetra ($n = 27$), and the Guadalupe roundnose minnow ($n = 15$) were associated with sites that had cooler temperatures and slightly higher conductivity. Another cyprinid species, the mimic shiner ($n = 10$), was collected in areas characterized by higher turbidity levels.

An analysis of similarity (ANOSIM) showed differences in community composition among periods (Table 4). The assemblages were significantly different between the drought and post-flood, and the recovery and post-flood periods (Bray-Curtis: $R = 0.087$, $P < 0.01$), and among all three periods (Jaccard: $R = 0.128$, $P < 0.05$). However, non-metric multidimensional scaling (MDS) failed to partition by period (Figure 5). This may be due to the high number of zero capture events not allowing for any distinct partitioning.

Macroinvertebrates – Overall - There was a total of 7,201 macroinvertebrates collected throughout this study (Appendix A-1), with 52 taxa represented from 17 orders. Within the class Hexapoda, eight orders, 23 families, and 36 genera were present in study sites across season. Fifty percent of all macroinvertebrates collected were hexapods. Among aquatic insect orders collected, Ephemeroptera (11%) were the most abundant, followed by Diptera (4%), Coleoptera (1%), Odonata (0.82%), Trichoptera (0.81%), and Lepidoptera (0.12%). The order Hemiptera (0.11%) was the least abundant. The most abundant non-hexapod orders were Gastropoda (44%), Amphipoda (32%), and Ostracoda (4%).

The most common taxon collected was the Thiarid snail, *Tarebia granifera*, which was present at all nine sites (at 90 of the 107 sampling events: 84%). Other common taxa with relatively high abundance include the amphipod *Hyaella azteca* (32%), *Tricorythodes* sp. (6%), Hydrobiidae (5%), Ostracoda (4%), Chironomini (2%), *Fallceon* (2%), *Melanoides tuberculata* (2%), and Orthoclaadiinae (1%). The eight least abundant genera all had one lone specimen and each contributed 0.01% of the total number collected and included *Stratiomis* sp., *Serromyia* sp., *Hetaerina* sp., *Stylurus* sp., *Microvelia* sp., *Dubiraphia* sp., *Macrelmis* sp., and *Heterelmis* sp. Total macroinvertebrates collected

varied significantly temporally ($F_{11,5604} = 2.279$, $P < 0.01$), however, total macroinvertebrates within algae (36%), *Riccia* (32%), and *Ludwigia* (32%) did not significantly vary spatially ($F_{8,5607} = 1.617$, $P = 0.114$) (Figure 12). The amphipod *H. azteca* was the most abundant macroinvertebrate collected in four of the nine microhabitats. These four microhabitats encompassed all three vegetation types, however, all four microhabitats where *H. azteca* was the most abundant were located within the impounded headwaters of the Comal River, Landa Lake. The gastropod *T. granifera* was the most abundant macroinvertebrate collected in the remaining five microhabitats, which were all located below Landa Lake (A - 4).

Macroinvertebrates – Focal Periods - A total of 3,740 macroinvertebrates were collected during the three study periods (Table 3). The drought period resulted in the most macroinvertebrates collected ($n = 1829$), highest Simpson's diversity ($\Delta = 0.5$), lowest richness (26), and lowest Pielou's evenness ($J = 0.63$). The most abundant macroinvertebrates collected were *T. granifera* (41%), *H. azteca* (37%), Cyprididae (3%), *Melanoides tuberculata* (3%), *Psephenus* sp. (2%), *Fallceon* sp. (2%), and *Elimia comalensis* (2%). The recovery period was the second highest in specimens collected ($n = 1459$), richness (27), Simpson's diversity ($\Delta = 0.34$), and Pielou's evenness ($J = 0.63$). The most abundant macroinvertebrates collected during the recovery were *H. azteca* (32%), *T. granifera* (18%), *Tricorythodes* sp. (17%), *E. comalensis* (5%), Orthoclaadiinae (4%), and Cyprididae (4%). The post-flood period had the fewest macroinvertebrates collected ($n = 452$), and lowest Simpson's diversity ($\Delta = 0.32$) but had the highest richness (30), and Pielou's evenness ($J = 85$). The most abundant macroinvertebrates collected post-flood were

Tarebia granifera (27%), *H. azteca* (23%), Cyprididae (4%), *Psephenus* sp. (4%), *E. comalensis* (3%) and *Palaemonetes* sp. (3%).

The first two axes on the macroinvertebrate CCA (Figure 4a) accounted for 21% of the total variation explained by the model. Twelve percent of the total variation was explained by CA I, whereas CA II explained an additional nine percent of the total variance in community composition. CA I described a gradient from the negative loadings of pH (-0.26), dissolved oxygen (-0.09), and current velocity (-0.08), to the positive loadings of temperature (0.19) and average depth (0.13). CA II was primarily described by the positive loadings of dissolved oxygen (0.28), depth (0.07), and pH (0.06), while the negative loading was current velocity (-0.003).

The more generalist species plotted out near the origin of the CCA species bi-plot (Figure 4b) included gastropods *T. granifera*, *M. tuberculata*, *E. comalensis* and *Marisa cornuarietis*; crustaceans *Palaemonetes* sp., *H. azteca*, and *Oronectes* sp.; and several odonates (calopterygidae and libellulidae), mayflies (Ephemeraeidae and Leptohyphidae), midges (Chironomini and Tanypodinae) and riffle beetles (Elmidae). As this is a spring fed system, it is not uncommon to see the majority of the macroinvertebrates plot out near the origin, however there are a few exceptions. *Nectopsyche* was collected in areas characterized by cooler temperatures and higher dissolved oxygen, whereas, *Macrelmis*, *Orthoclaadiinae*, and *Petrophila* were collected in areas characterized by low dissolved oxygen and warmer temperatures.

Analysis of similarity (ANOSIM) showed significant differences in macroinvertebrate assemblage by period (Table 4). Pair-wise tests showed there were differences between all three focal periods: drought – post-flood, drought – recovery, and

post-flood - recovery (Bray-Curtis: $R = 0.164$, $P = 0.001$). Non-metric multidimensional scaling (MDS) did not produce separation in space among periods (Figure 6), however, there was slight grouping among individual microhabitats.

Fountain darter diet and electivity

A total of 222 fountain darters from Comal Springs were included in diet analysis (Site 1L = 7; Site 1A = 28; Site 2B = 52, Site 3B = 49; Site 4B = 16; Site 4A = 8; Site 4L = 50; Site 5L = 10; Site 5A = 13) consisting of 116 females, 105 males. A total of 1,110 prey items were identified from the digestive tracts, with 22 fountain darters having empty digestive tracts.

Hyaletta azteca, which made up 32% of the macroinvertebrate community sampled overall, and was present at all sites, was the most common taxon found in the digestive tract and benthic samples (Figure 15). There were no significant differences in fountain darter diet spatially ($F_{8,213} = 1.485$, $P = 0.164$) or temporally ($F_{11,210} = 0.811$, $P = 0.629$). However, the differences in number of diet items contained in the stomach was found to be significantly different among periods ($F_{2,264} = 5.575$, $P < 0.01$), with digestive tracts during the drought averaging 5.4 items versus 4.0 during the recovery, and 2.7 post-flood.

During the drought, *H. azteca* comprised the majority of diet items found in the digestive tract (30%), followed by ostracods (Cyprididae 26%), calanoids (Calanidae 14%), and midges (Chironominae 10%). Collectively, these taxa comprised 79% of the diet of fountain darters examined during the drought. The percent composition of dietary prey items collected was similar to the frequency of occurrence in the environment (Spearman's $\rho = 0.940$, 2 d.f., $p < 0.05$) (Figure 15). During the recovery period, there was a shift in

prey items with the presence of mayflies; however, *H. azteca* remained the major taxa found in the gut (46%), followed by Cyprinids (25%), *Tricorythodes* sp. (9%), and *Baetis* sp. (7%). The percent composition in the digestive tracts of fountain darters during the recovery period was not significantly different than the frequency of occurrence of macroinvertebrates found in the benthic samples (Spearman's rho = 0.875, 2 d.f., p = 0.12). There was consistency in the diet during the post-flood period, with *H. azteca* (39%) continuing to be the most abundant prey item, followed by Cyprinidae (14%), *Baetis* sp. (13%), and midge Tanytarsini (10%). The percent composition of prey items during the post-flood period mirrored the frequency of occurrence in the environment, and was not found to be significantly different (Spearman's rho = 0.784, 2 d.f., p = 0.21).

Analysis of similarities (ANOSIM) showed a significant difference (Bray-Curtis: R = 0.098, P < 0.001; Jaccard: R = 0.101, P < 0.01) in diet composition among periods (Table 4). Pair-wise comparisons displayed significant differences in diet items among all three disturbance periods. Non-metric multidimensional scaling (MDS) did not present separation among periods (Figure 7).

Strauss' linear index (L_o) indicated no apparent electivity or avoidance of prey items among the three periods (Table 5). Fountain darters exhibited a slight positive selection towards Cyprinids during both drought ($L_o = 0.22$; n = 40) and recovery ($L_o = 0.20$; n = 34) periods, and towards *H. azteca* ($L_o = 24$; n = 14) during the post-flood period. Fountain darters exhibited the lowest selection towards Calanids, *Baetis* sp., and Tanytarsini during drought, recovery, and post-flood respectively, indicating near random selection of these prey items. Although there were no strong selectivity scores, there were no avoidance scores among the three periods within the top four prey items in each period. Within the drought

period, fountain darters appeared to exhibit slight selection towards prey items that were not as abundant in the environment (Figure 16). This behavior is mirrored during the recovery period (Figure 17). During the post-flood period, there appeared to be an inverse relationship between percent prey items in the environment and percent prey items in digestive tracts (Figure 18).

Gill parasites

Significant results were found in infection of the fountain darters by *Centrocestus formosanus* cyst densities spatially ($F_{8,213} = 2.952$, $P < 0.01$) with uppermost Landa Lake site 1L having the fewest average cysts per fish (12.8 ± 5.0) versus the lowermost Landa Lake site 3B, averaging the highest number cysts per fish (70 ± 10.4) (Figure 20b). Within microhabitats, *Riccia* averaged 53.4 cysts per fish compared to 40 in algae and 32.8 in *Ludwigia*, although these differences were not found to be significant. Among the three periods, significant differences were also found in cyst densities with the drought period having the highest average densities, followed by the post-flood period, and the recovery period having the lowest average densities ($F_{2,133} = 3.305$, $P < 0.05$; Figure 21).

A multiple regression was performed using seven water quality variables (temperature, pH, conductivity, dissolved oxygen, turbidity, depth, and current velocity) against cysts densities to evaluate if these significantly influenced cyst densities. Only two variables, current velocity and turbidity, were shown to be significant, however only current velocity was selected for further analysis as turbidity was believed to be influenced by recreational effects. The furthest downstream sites (4 and 5) were most prone to turbidity from recreational influence, with site 4 being comprised of the Old Channel of the Comal

River that is separated from the main stem by two weir dams and site 5 which is located in the main stem itself. Current velocity was then used in a linear regression to evaluate its influence on cyst densities (Figure 19) with weakly significant results ($R^2 = 0.018$, $P = 0.001$).

DISCUSSION

Headwater spring systems are often hydrologically and thermally stable and show little fluctuation as far as abiotic variables are concerned (Fritz, Tripe, and Guy 2002). Similarly, the nearby spring-fed San Marcos River headwaters exhibit very low variability in chemical and physical characteristics (Groeger *et al.* 1997). This was evident throughout the majority of the sampling year for this study with the exception of the two disturbance periods. Although physicochemical and vegetative properties changed among our disturbance periods, fountain darters were observed and collected at all sites because this is a spring fed system, abiotic characteristics are fairly stable and fountain darters are found throughout, exhibiting no preference to areas described by specific abiotic variables. On the CCA species bi-plot (Figure 3b), the fountain darter plotted out near the origin, representing its cosmopolitan distribution within the system. Collected spatially throughout the study system, the fountain darter was present year round at all five sites and during all three periods. The inherent thermal and physicochemical stability of Comal Springs is likely what designates the fountain darter as a generalist species within this system, but does not allow it to disperse out of the headwaters into the Guadalupe River.

The fountain darter displayed microhabitat preference. Three microhabitat types were examined, *Riccia fluitans*, *Ludwigia repens*, and *Rhizoclonium* sp., with the most fountain darters collected in the *Riccia* habitat. It has been shown in previous studies

(Schenck & Whiteside 1976; Linam *et al.* 1993) darters prefer low growing vegetation as it is an optimal habitat for egg deposition (Roberts & Angermeier 2007, Duncan *et al.* 2010, Phillips *et al.* 2010) and foraging (Duncan *et al.* 2010). We observed a similar relationship with the fewest fountain darters collected in *Ludwigia* and the largest number of total fountain darters collected in low growing *Riccia*. Several spring darter species, the watercress darter (Duncan *et al.* 2010), the least darter (Seilheimer and Fisher 2010), and the cold-water darter (Lang and Mayden 2007) are associated with low growing bryophytes.

Further possible explanations for habitat preference by darters include presence of predators. Schlosser (1987) observed darters and other small bodied fishes were restricted to shallow refugia in the presence of large bodied centrarchid predators. The low growing nature of *Riccia* restricts its distribution to shallower depths within riverine systems and may account for higher fish densities. Shallow refuge is not the only behavioral adaptation observed in fishes avoiding predators. Stuart-Smith (2008) observed populations of the golden galaxias (*Galaxias auratus*), normally an open water inhabitant, occupying densely populated areas of macrophytes in the presence of the brown trout (*Salmo trutta*). Savino & Stein (1982) found the effectiveness of predation by largemouth bass was tied to habitat complexity. As vegetation became more complex, foraging was negatively affected due to diminished visual contact with potential prey. In addition to habitat complexity, current velocity plays an integral role in predation by large bodied centrarchids. Brewer (2011) found in times of decreased current velocities, smallmouth bass selected habitats in shallower depths. Braun and Walser (2011) found the lack of slow moving waters limit the number of largemouth bass in a river system. We have determined fountain darters were more abundant within the *Riccia*, which itself is present at decreased depths and velocities.

The likelihood of these centrarchids inhabiting shallower waters in times of reduced current velocity could lead to higher incidences of predation on the fountain darter in times of drought.

The seasonal increase in temperatures and decreased water levels during the drought changed the aquatic vegetative landscape, causing stands of macrophytes to decrease or even disappear, so any available habitat may have been occupied by fishes. Parkos *et al.* (2011) found in their study of everglade assemblages, that fishes, particularly during drought episodes, sought out available habitat to avoid being preyed upon. Therefore, suitable microhabitat patches would potentially have served as refugia for darters, resulting in more darters occupying available stands leading to the larger densities. We found similar results with the highest number of fountain darters collected in available habitat during the drought and the fewest total darters collected occurred during the initial recovery following a large rain event (October 2009). The high fountain darter densities were likely the result of colonization of available habitat. The drought period likely concentrated darter densities into available habitat, similar to the findings by Davis and Cook (2010) where drought periods led to crowding in available microhabitats. The drought period resulted in the highest densities of darters captured, followed by the recovery period, and lastly the post-flood period resulted in the lowest densities. The recovery period resulted in large amounts of uncovered *Riccia* throughout the system. It is possible that fountain darters dispersed among a greater abundance of appropriate habitat resulting in fewer darters being captured. In a study of recolonization of warm water fishes, Adams and Warren (2005) observed recolonization of fishes as a highly ordered, non-random process that was dictated by the resumption of river flows, and as river discharge ultimately dictates macrophyte growth,

discharge dictated recolonization. Fish incrementally accumulated over time in newly available habitat from their refugia, highlighting their role as source colonizers (Adams and Warren 2005). The flood occurred in June resulted in the streambed being scoured by floodwaters, thus displacing macrophytes and denuding areas throughout the Comal system. Fountain darters were collected in bare substrate, (that is cobble/gravel with no vegetation), during sampling efforts after the flood period, however, densities were far less than efforts pre-flood.

Community structure and composition

Fish assemblage – We predicted to find the highest abundance of fish during our recovery period, however the highest fish abundance was observed during the drought period. We attribute highest fish abundance during this period due to populations being concentrated in available habitat. In a review of the role of fish refugia during drought, Magoulick and Kobza (2001) found that during drought, if barriers were absent, fishes moved into available habitat, increasing densities, therefore causing a concentration effect. Furthermore, those fishes that find suitable refuge are best characterized by source-sink dynamics, as these fishes within the newly inhabited refuge are now part of a source population that could eventually colonize new patches (Magoulick and Kobza 2001).

Schlosser and Angermeier (1995) discuss five different classes of metapopulation models (classic, source/sink, patchy, hybrid, and non-equilibrium) that differ in spatial variation regarding reproduction, extinction, and colonization. The classic metapopulation model is described by several similarly sized populations, all subject to extinction with equal and temporally independent probabilities that persist due to recolonization from

adjacent populations. The source/sink model is described by a large mainland population that normally is the origin of immigrants to outlying populations. The patchy model is similar to the classic model, differing in a high dispersal between habitat types of habitat patches. The hybrid model combines characteristics of the source/sink with characteristics of the patchy models. The non-equilibrium model emphasizes the importance of local extinction and infrequent recolonization. Of these models, it is the “mainland/island or source-sink” metapopulation model that best describes Comal Springs during the drought period. Optimal habitat decreased or disappeared throughout Comal Springs, and as the drought intensified, the smaller patches were eliminated leaving only the largest patches. These larger patches were likely continually colonized by fishes and assumed the role of the ‘source’ or ‘islands’ within the system. The source/sink model differs from the classic model in that one or more of the populations has greater potential for providing emigrants than the other populations. Pulliam (1988) suggests lotic system fish populations are best described by source-sink models. In these models, source populations produce excess individuals that would recolonize sink habitats. Furthermore, Pullam (1988) argues habitat patches that are highly occupied represent higher quality habitat, as organisms occupy habitat until all breeding sites within a patch are taken, and only until then will there be emigration by organisms with higher fitness to sink habitats. This however describes populations during a state of equilibrium, and does not account for disturbance periods. It is likely during disturbance periods those organisms that emigrate from the source patch seemingly would not persist in the sink patch as there would be greater probability the patch itself would not survive the disturbance, thus affecting reproductive fitness. This argument does support colonization patterns upon succession following a disturbance period, where

organisms emigrate from source patches to sink patches in efforts to find optimum breeding habitat. This could explain the observations we saw with fish and macroinvertebrate abundances between drought and recovery periods, where abundance was highest during the drought, and decreased during the recovery period, when vegetation patches returned throughout the system. In a review of source-sink dynamics, Diffendorfer (1998) highlights dispersal is generally regulated by mechanisms that maximize fitness, commenting that most observations regarding source-sink communities operate under the rule of not occupying a patch if a better one is available.

Shifts in fish abundances were noticed among periods, particularly with those fishes that were tolerant to eurythermal conditions. In times of drought, fishes that favored eurythermal conditions may have gained habitat at the expense of fishes that favor stenothermal conditions. During the drought, the fountain darter and both *Gambusia* species (*G. affinis* and *G. geiseri*) were collected in high densities, however during the recovery period, the *Gambusia* species were collected in far fewer numbers. Hubbs (2003) observed eurythermal *G. affinis* and *G. senilis* gained area at the expense of spring endemics (*G. nobilis*, *G. gaigei*, *G. hurtadoi*, and *G. alvarezzi*) during decreased watering events. Perkin *et al.* (2012) evaluated life history aspects of the ironcolor shiner (*Notropis chalybaeus*) in the spring-fed headwaters of the San Marcos River and observed its distribution is limited to the upper 2.2km of the San Marcos River, which exhibits stable characteristics (e.g. temperature, pH) of spring-fed riverine systems. During a separate mark-recapture study, habitat previously inhabited by the ironcolor shiner was diminishing due to dewatering and increased temperatures, and no collections were made of *N. chalybaeus* (Shattuck, unpublished data), but did result in increased collections of centrarchids and *Gambusia spp.*

Hubbs and Springer (1957) observed the co-occurrence of the ironcolor shiner with the largespring gambusia, which is considered a stenothermal fish. Like the ironcolor shiner, the fountain darter's distribution is likely tied to stenothermal conditions, and during times of drought these conditions are susceptible to fluctuations, further limiting distribution of the fountain darter, or encouraging distribution of fishes with wider abiotic tolerances.

Conversely, we see the fewest fish being collected during our post-flood period, after available habitat was scoured from the system.

Species richness was greatest during the drought period, with 11 species observed, followed by the recovery period with 10 species, and the post-flood period with five species observed. Simpson's diversity scores indicate the recovery period as having the highest diversity, being composed mostly by the mimic shiner, sailfin molly, and the fountain darter. The drought period was dominated by the western mosquitofish, redspotted sunfish, Texas shiner, and the fountain darter, and the post-flood period was the least diverse characterized mainly by bluegill and the western mosquitofish. Adams and Warren (2005) conclude after drought disturbance, fish diversity is lower, then increases at a considerable rate, due to immigration and reproduction. McCargo and Peterson (2010) evaluated seasonal base flow influence on fish assemblages and concluded base flow volumes were positively related to fish density and species richness, observing highest fish captures and species richness during low base flow (drought) periods.

Similarities in community composition were characterized by species abundance, species richness, species diversity, and species evenness. Community composition was different when comparing the post-flood period to the recovery and drought periods, with community composition being similar between the recovery and drought. We hypothesized

community composition would be greatest during the recovery period, but we found it to be greatest during the drought period. The similarities between recovery and drought community structure may be attributed to suitable habitat being available, and the dissimilarities with the post-flood period were likely due to a lack of suitable habitat that would otherwise provide shelter from being displaced during a flood period.

Macroinvertebrate assemblage – Community composition was represented by five major groups; the gastropods, amphipods, mayflies, dipterans, and ostracods. Snail taxa (*T. granifera*, *M. tuberculata*, *E. comalensis*, *M. cornuarietis*, *Stenophysa sp.*, and Hydrobiids), mayflies (*Caenis sp.*, *Tricorythodes sp.*, *Hexagenia sp.*, and *Leptohyphes sp.*), amphipod (*H. azteca.*), and flies (Tanypodinae and Chironomini) were found throughout the system and were not strongly associated with any environmental variable, qualifying them as cosmopolitan groups within this system.

We observed a similar pattern in macroinvertebrate densities as we did with fishes, with the drought period resulting in the highest abundance of macroinvertebrates collected and the post-flood period with the lowest abundance. Stubbington *et al.* (2009) observed an inverse relationship with flow permanence and macroinvertebrate abundance, stating as stream flows reduced, Oligocheate and Chironomid abundances flourished. It is important to note, although macroinvertebrate abundances were highest during the drought, there was not a dramatic reduction between drought and recovery. Predation, or lack thereof, could also have accounted for a relatively high macroinvertebrate abundances during the recovery event. Dorn (2008) found macroinvertebrate abundances were high after a rewetting period in Florida wetland ponds following drought episodes that depleted fish populations. The absence of predatory fishes allowed macroinvertebrates to persist in those ponds, and as fish

abundance was significantly lower during our recovery period, this could also explain why macroinvertebrate abundances were high. Fritz *et al.* (2002) observed after a flood in a Kansas stream, that macroinvertebrate abundance was reduced by more than 99%, and diversity reduced by more than 95%. Moorhead *et al.* (1998) examined the succession of macroinvertebrate communities following rewetting events and observed macroinvertebrate recolonization began slowly, dominated by crustaceans and filter feeders, but rapidly was matched by herbivores and predacious insects. They conclude by observing species richness, diversity, and community similarity in composition followed a pattern of progressive colonization by species capable of exploiting rewetted conditions.

Macroinvertebrate diversity was different when comparing the three periods. A likely driver in community structure is the availability of habitat in general throughout this study, with populations being concentrated in small areas of habitat present during the drought, and with vastly decreased numbers following the flood period. The seasonal nature of aquatic macroinvertebrates also plays a role. In their study of resistance and resilience of macroinvertebrates to drying and flooding streams, Fritz and Dodds (2004) found macroinvertebrate abundances were highest during the lowest flows, attributing this to the decline in area of submerged habitat. Stubbington *et al.* (2009) also observed *Gammarus* species utilizing substrate clasts as refuge over surficial sediments during seasonal drying episodes, as the clasts provided better shelter. Both the drought and post-flood periods occurred during the traditional summer season, during or after the period when most macroinvertebrates emerge as adults, therefore it is not surprising to see species richness being low during these two periods. Our recovery period occurred during spring months, during a time when emergence into aquatic stages is most often occurring, which is likely

what explains species richness being greatest during this time. High species richness during the recovery period is likely due to life history characteristics of the macroinvertebrates that are characterized by non-seasonal life cycles. Non-seasonal life cycles are characteristic of macroinvertebrates found in systems that are subjected to unpredictable disturbance (Merritt and Cummins 2008).

Diet analysis

The major constituents of fountain darter diet were amphipods, ostracods, calanoids, and mayflies. Bergin (1996) found the fountain darter to be a generalist predator, often feeding on microcrustaceans. Five out of the top six macroinvertebrate families found (by frequency of occurrence in digestive tracts) in the 1996 study were also found to be the most abundant in this study. The main prey consumed by the fountain darter are relatively mobile in the benthos, which probably results in their being consumed, as the fountain darter forages based on visual cues by prey movement. Electivity scores indicated no strong selection towards any particular prey item, nor did they indicate strong avoidance, with scores indicating near random feeding patterns. Overall, we saw the percent composition in the digestive tracts mirror the frequency of occurrence in the environment, with *H. azteca* being the most abundant, having been found in 32% of benthic samples and comprising 37% of overall prey items. With its fairly large presence in the diet and distribution in the environment, *H. azteca* exhibited an electivity score close to zero, indicating random selection. Strange (1993), Knight and Ross (1994) and Alford and Beckett (2006) found chironomids to be the most abundant prey item in fantail darter, bayou darter, and speckled darter digestive tracts, respectively, while concurrently making up a large proportion of the resource base.

Although we anticipated a pattern between diet items present in the gut and percent abundance in the environment, this pattern was weak, if present, during the three periods. For example, cyprididae and chironomini comprised a fair portion (14% and 10%) of the gut contents during the drought period, but only constituted a fraction (0.5% and 0.05%) of total benthic invertebrates collected. This inverse relationship between abundance in gut with abundance in the benthos has been seen in other systems where darter species are distributed. Henry and Grossman (2008) found in a study of microhabitat use by three darter species during drought that the darter species did not select microhabitats with higher prey abundances. Alford and Beckett (2007) observed the diets of four darter species, the brighteye darter, the speckled darter, the gulf darter, and the black banded darter, were comprised of prey items that were not abundant in the environment on a species level.

Temporal changes were also observed with prey items mirroring abundance in the environment. Examples include a greater number of chironomids in the environment and diet during both the post-flood and drought periods. These periods occurred during the late summer months, which have been shown to be peak chironomid emergence times. Similarly, the mayflies are represented in both the diet and environment during spring months, which coincide with the recovery period. Ostracods were present among the three periods however they comprised a greater proportion in the diet than they did in the environment. Alford and Beckett (2006) found microcrustaceans, primarily cladocerans, to comprise a fair portion of the speckled darter's diet, however the percent abundance in benthic samples was low. They attributed this disparity to be due to the epibenthic nature of the cladoceran species.

Analysis of similarities displayed a difference in diet makeup among the three disturbance periods. Subsequent pairwise comparisons revealed significant differences in diet items among all three periods. This is not unexpected as macroinvertebrates have seasonal influences with regards to reproduction, larval stage duration, and emergence times (Merritt and Cummins 2008). Also contributing to the differences among diet items would be the availability of habitat, or lack thereof, that would inhibit adult macroinvertebrates from depositing their eggs within the individual microhabitats.

Gill parasites

We saw the most cysts present during the drought period. Mitchell and Brandt (2005) and Fleming *et al.* (2011) found the primary host, *M. tuberculata*, to persist within a temperature range between 18 - 32°C. Mitchell and Brandt (2005) also reported the finding of *C. formosanus* by Utah Division of Wildlife Resources personnel in two geothermal springs in Utah, indicating its viability at warmer temperatures. The intermediate cyst densities found during the recovery period are likely an artifact of the stenothermal nature of Comal springs. The fewest cysts were observed during the post-flood period. This could be a result of the cercariae simply being flushed downstream due to the flood pulse, reducing the number of cercariae present to infect present fountain darters. Destruction of cercariae due to the turbulent nature of the post-flood period was also likely. Lozano (2005) observed cercarial concentrations decreased spatially due to natural mortalities, as well as loss due to riffle turbulence. The flood pulse could have provided riffle like consequences on a larger scale.

The site located within the impounded headwaters, Landa Lake, had the highest average cysts per fish. Mitchell *et al.* (2000) found very dense populations of infected snails

within Landa Lake. In his evaluation of metacercarial densities in fountain darters throughout the Comal River, Cantu (2003) also found infected darters to have as many as 1,600 cysts per fish within Landa Lake. The high cyst numbers associated with the Landa Lake site could be attributed to the propensity of wading birds to congregate in or around the area. Mitchell *et al.* (2000) suggest the yellow-crowned night heron, *Nyctanassa violacea*, is the definitive host for the *C. formosanus*, while Kuhlman (2007) suggests the definitive host is the green heron, *Butorides virescens*. Both of these bird species are present in Comal Springs at various times throughout the year. Our farthest upstream site averaged the fewest cysts per fish. This site was located in a residential area where wading birds are less frequently observed. Fleming *et al.* (2011) were unable to document presence of *M. tuberculata* in the lower reaches of the Comal River. During this study, we were able to collect *M. tuberculata* at our most downstream site, indicating possible expansion of the melania snail's range.

We also had a positive correlation between increased spring discharge and cyst densities. Cantu (2003) found there to be a negative correlation between spring discharge and cyst densities. Our relationship between spring discharge and cyst densities was weak, therefore we cannot conclude with any confidence that this current velocity positively influenced cyst densities.

Conservation Implications

This study was the first to characterize fountain darter habitat and associated assemblages during and following a drought. Although there have been several studies involving the fountain darter, none have taken a comprehensive ecological approach. While other studies have focused solely on a single aspect, this study sought to evaluate multiple

ecological dimensions and their response to disturbance. This study evaluated how community composition and structure, habitat associations, diet, and parasite infection levels were affected by a drought period. Our findings indicate concentrated densities in fountain darters, fish and macroinvertebrate assemblages, diet items, and parasite cysts all associated with the low flows of the drought. The increased concentrations emphasize the concern over maintaining healthy populations of the fountain darter under drought conditions, and possibly highlight the threat to persistence during periods of extended drought.

We have found that fountain darters are distributed throughout the Comal Springs and River system in high densities year round; however, their distribution is likely limited to availability of habitat. This is highlighted by the flood that resulted in the high flows denuding the streambed of vegetation. This temporary destruction of habitat was felt across all trophic levels as there were fewer invertebrates, fewer darters, and fewer fish collected during the post-flood period. Furthermore, the drought disturbance could also have deleterious effects on darter densities. Although presented in this study as having the highest fish and macroinvertebrate abundance and richness, the drought period was characterized by a reduction of optimal habitat, leading to localized concentrations of both fishes and macroinvertebrates. Albanese *et al.* (2004) found no pervasive effects by drought on movement of warm water fishes in Virginia streams, indicating site fidelity. Additionally, Dammeyer (2010) studied movement patterns of the fountain darter within the Comal River. He concluded the fountain darter exhibited high site fidelity, particularly within the *Riccia* microhabitat. This site fidelity could be detrimental in times of extended drought, whereby habitat could be reduced further, displacing resident fishes, reducing prey items, and

decreasing possible shelter from predators. Cessation of flows could likely cause extinction of the fountain darter within the ecosystem under the most severe drought scenario.

Taking into account the abundance and richness of both fish and macroinvertebrate assemblages between the drought and recovery periods, it is likely the Comal Springs system exhibits those qualities that characterize a very stable system defined by a supra-seasonal disturbance period (low to moderate resistance and variable resilience). Although abundance and richness were greater during the drought period, similarity analyses indicated similar community makeup between the two periods. This would lead us to two possible explanations: 1) the system was able to resist changes brought about due to disturbance, or 2) the system exhibited resilient qualities that allowed it to recover from disturbance. The second scenario is more likely to have occurred as we have documented clear changes between the two periods. It is unclear how to characterize Comal Spring's stability as a system regarding its response to flood periods, as all sampling ceased a month after this period.

Conservation efforts during times of drought are necessary to maintain populations of the fountain darter. Defining drought conditions for Comal Springs will be difficult as drought indices (SPI, PDSI) are often measured in terms of ground moisture. Although ground moisture is an important factor for terrestrial systems, its applicability in aquatic systems seems misplaced. The term drought often leads to an image of a dying field or dry creek bed, so when a river is flowing, it may be difficult for people understand that although there may be water in a river, the system too can be affected by a terrestrial drought, even before drying events occur. Further complicating the issue of assigning drought to aquatic systems is the need for water for human use. Municipal and agricultural needs are primary

reasons for water extraction, and managing water resources often favors human needs relative to ecosystem conservation.

Conservation and recovery of the fountain darter must first begin with identifying the problem and accepting that a threat exists. Secondly, evaluation of trends leading up to and after the threat need to be conducted. Based on observed trends, actions must be taken to ameliorate the problem. We have identified a problem and potential threat to fountain darter populations in times of drought. Reduced habitat within the system during drought appears to be a significant threat to fountain darter populations. Ensuring the presence of optimal habitat needs to be a priority when implementing conservation strategies to ensure persistence of the fountain darter. Should optimal habitat not be present within the system, we would predict displacement of the fountain darter would occur, subjecting populations to increased predation, competition with other organisms for resources, decreased reproduction, and possibly a substantial decrease in overall population numbers or extirpation.

Results of this study can aid environmental stewards in developing management plans that take common disturbances into account. Decreased spring flows may be more of a threat to conserving this species than previously believed, as fish abundance has been shown to be greater in areas with higher spring discharge (Fritz *et al.* 2002). Aside from complete cessation of spring discharge, this study has shown that drought or drought like conditions (in the form of decreased water levels), threatens habitat availability which ultimately threatens fountain darter populations.

Table 1 Results from ANOVA test by season for water chemistry data with mean (+ SE).

Season	Temp (°C)	pH	Cond (μ S/cm)	D.O. (mg/L)	Turbidity (NTU)	Depth (m)	Velocity (m/s)
Drought	24.20(0.3)	7.23(0.1)	557(0.0)	7.65(0.3)	7.21(3.1)	0.76(0.1)	0.05(0.0)
Recovery	22.96(0.3)	7.14(0.0)	557(0.0)	5.88(0.2)	1.01(0.2)	0.78(0.1)	0.10(0.0)
Post-flood	23.91(0.1)	7.47(0.1)	564(0.0)	6.29(0.2)	1.15(0.2)	0.83(0.1)	0.11(0.0)
P	<0.001	<0.01	562	<0.001	<0.05	0.872	0.236

Table 2. Relative abundance (%) of fish species, taxa richness, Simpson's diversity, and Pielou's evenness of fish collected during drought, recovery, and post-flood periods.

Species	Period			
	Drought	Recovery	Post-Flood	Total
<i>Lepomis megalotis</i>	0.4	0.2	-	0.2
<i>Lepomis macrochirus</i>	<0.1	0.3	2.9	1.1
<i>Lepomis miniatus</i>	1.3	2.8	-	1.4
<i>Cichlasoma cyanoguttatum</i>	0.6	0.1	-	0.2
<i>Notropis amabilis</i>	0.3	-	-	0.1
<i>Notropis volucellus</i>	0.2	-	-	<0.1
<i>Dionda nigrotaeniata</i>	<0.1	0.2	-	<0.1
<i>Etheostoma fonticola</i>	8.5	36.0	44.1	29.6
<i>Etheostoma lepidum</i>	0.1	-	-	<0.1
<i>Gambusia affinis</i>	87.7	57.9	49.7	65.1
<i>Gambusia geiseri</i>	0.3	-	-	0.1
<i>Poecilia latipinna</i>	0.2	2.0	-	0.7
<i>Ameiurus natalis</i>	-	0.1	0.2	0.1
N=	2,824	580	412	3,816
Taxa Richness	11	10	5	26
Diversity	0.68	0.73	0.62	
Evenness	0.48	0.47	0.36	

Table 3. Relative abundance (%) of macroinvertebrate species, taxa richness, Simpson's diversity, and Pielou's evenness of macroinvertebrates collected during drought, recovery, and post-flood periods

Species	Period			
	Drought	Recovery	Post-Flood	Total
<i>Baetis</i>	0.4	1.0	2.7	1.4
<i>Callibaetis</i>	0.8	0.4	1.9	1.0
<i>Falceon</i>	1.6	2.6	1.5	1.9
<i>Tricorythodes</i>	0.1	16.7	2.2	6.3
<i>Caenis</i>	0.7	-	0.2	0.3
<i>Hexagenia</i>	1.1	1.1	-	0.7
<i>Chironominae</i>	<0.1	2.0	8.4	3.5
<i>Orthoclaadiinae</i>	-	3.7	0.6	1.4
<i>Tanypodinae</i>	0.2	0.4	2.2	0.9
<i>Tanytarsini</i>	-	1.9	1.1	1.0
<i>Serromyia</i>	-	<0.1	-	<0.1
<i>Argia</i>	0.3	-	0.7	0.3
<i>Hetaerina</i>	-	-	0.4	0.1
<i>Helicopsyche</i>	1.1	<0.1	1.5	0.9
<i>Oecetis</i>	0.2	-	-	<0.1
<i>Hydropsyche</i>	-	<0.1	0.2	<0.1
<i>Nectopsyche</i>	-	-	0.7	0.2
<i>Metrobates</i>	<0.1	-	-	<0.1
<i>Trepobates</i>	-	-	0.2	<0.1
<i>Hyaella</i>	37.3	32.1	22.8	30.7
<i>Psephenus</i>	2.3	0.1	3.5	1.9
<i>Heterelmis</i>	-	-	0.2	<0.1
<i>Macrelmis</i>	-	<0.1	-	<0.1
<i>Microcylloepus</i>	0.2	<0.1	0.4	0.2
<i>Paraponyx</i>	0.1	-	-	<0.1
<i>Oronectes</i>	<0.1	1.0	0.9	<0.1
<i>Palaemonetes</i>	0.2	1.8	3.1	1.7
<i>Melanoides</i>	2.9	1.8	1.5	2.0
<i>Tarebia</i>	41.2	18.2	27.2	28.9
<i>Hydrobiidae</i>	1.8	5.1	1.5	2.8

Table 3 - Continued

Species	Period			Total
	Drought	Recovery	Post-Flood	
<i>Elimia</i>	1.8	4.6	3.1	3.2
<i>Helisoma</i>	-	0.1	-	<0.1
<i>Marisa</i>	-	0.1	0.2	0.1
<i>Stenophysa</i>	-	<0.1	0.4	0.2
<i>Corbicula</i>	-	-	0.2	<0.1
<i>Calanidae</i>	<0.1	-	-	<0.1
<i>Cyprididae</i>	3.4	3.7	3.5	3.5
<i>Hydrachnida</i>	0.2	0.6	-	0.2
<i>N=</i>	1829	1459	452	3,740
Taxa Richness	26	27	30	83
Diversity	0.50	0.34	0.32	
Evenness	0.63	0.63	0.85	

Table 4. ANOSIM global and pair-wise tests illustrating significance of fish and macroinvertebrate communities, and diet among periods.

Sampling Period			Bray Curtis		Jaccard	
			R	<i>P</i> value	R	<i>P</i> value
Fish Assemblage						
Global test			0.087	<0.01	0.128	<0.05
Pairwise tests:						
Drought	vs	Post-Flood	0.092	<0.05	0.133	<0.01
Drought	vs	Recovery	0.02	0.19	0.121	<0.01
Recovery	vs	Post-Flood	0.151	<0.01	0.135	<0.01
Macroinvertebrate Assemblage						
Global test			0.164	<0.001	0.103	0.09
Pairwise tests:						
Drought	vs	Post-Flood	0.217	<0.001	0.055	0.08
Drought	vs	Recovery	0.069	<0.05	0.133	<0.01
Recovery	vs	Post-Flood	0.209	<0.001	0.118	<0.01
Fountain Darter Diet						
Global test			0.098	<0.001	0.101	0.01
Pairwise tests:						
Drought	vs	Post-Flood	0.166	<0.001	0.168	<0.001
Drought	vs	Recovery	0.042	<0.05	0.041	<0.05
Recovery	vs	Post-Flood	0.114	<0.001	0.119	<0.001

Table 5. Strauss Electivity Scores (L_o) for a) drought, b) recovery, and c) post-flood periods showing maximum and minimum values with the number of fountain darters that consumed a specific prey item (n).

a)

Prey Items	L_o	Maximum	Minimum	n
Chironomini	0.08	0.67	-0.45	16
<i>Hyaella</i>	0.14	1	-0.62	40
Calanidae	0.09	0.92	0	14
Cyprididae	0.22	0.71	0.11	40

b)

Prey Items	L_o	Maximum	Minimum	n
<i>Tricorythodes</i>	0.11	1	-0.21	13
<i>Baetis</i>	0.05	0.67	-0.30	10
<i>Hyaella</i>	0.18	1	-0.38	23
Cyprididae	0.20	0.67	-0.13	34

c)

Prey Items	L_o	Maximum	Minimum	n
<i>Baetis</i>	0.08	1	-0.39	8
<i>Tanytarsini</i>	0.02	0.55	-0.11	2
<i>Hyaella</i>	0.24	1	-0.50	14
Cyprididae	0.11	1	-0.17	8

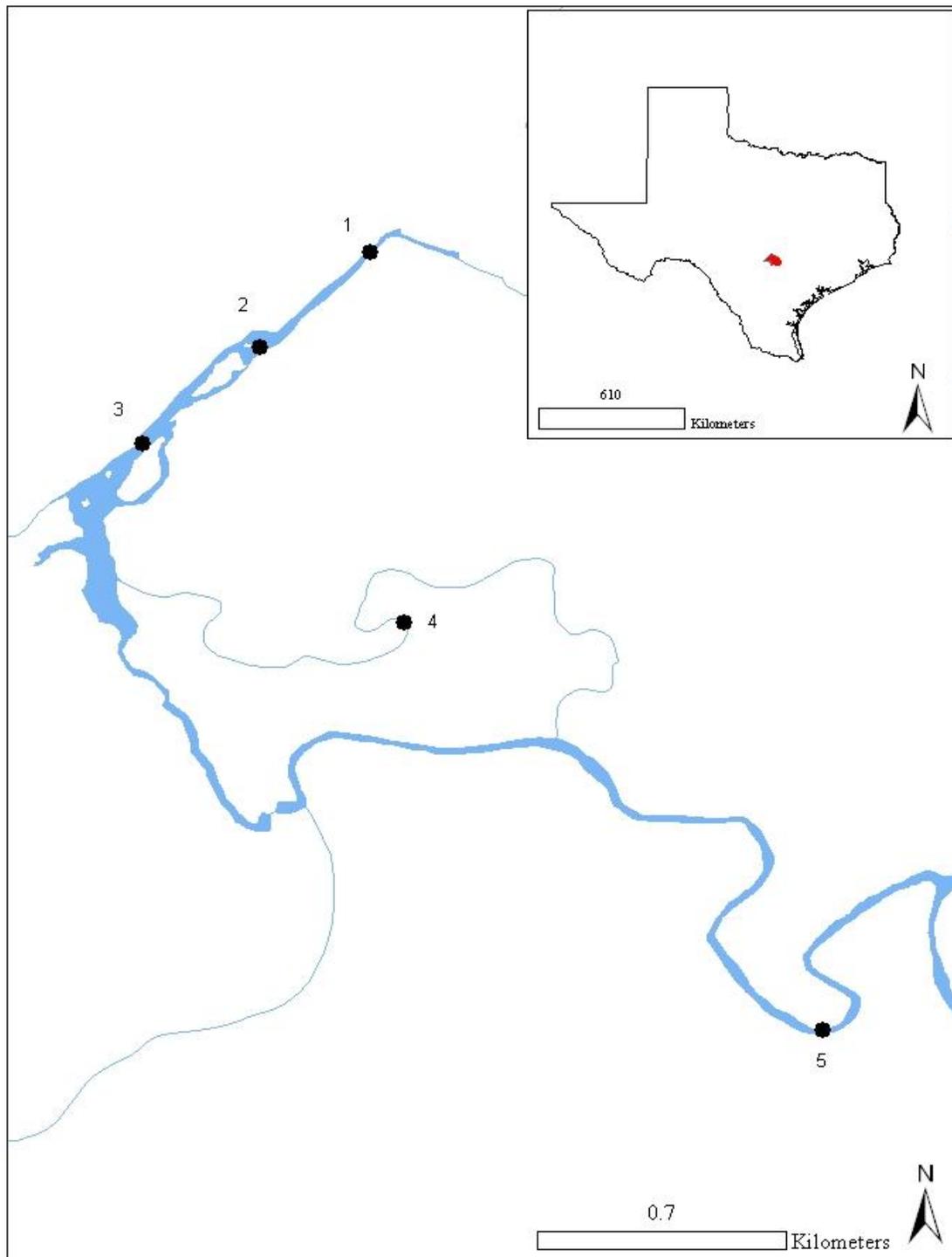


Figure 1. Sampling sites within the Comal River, Comal County, Texas.

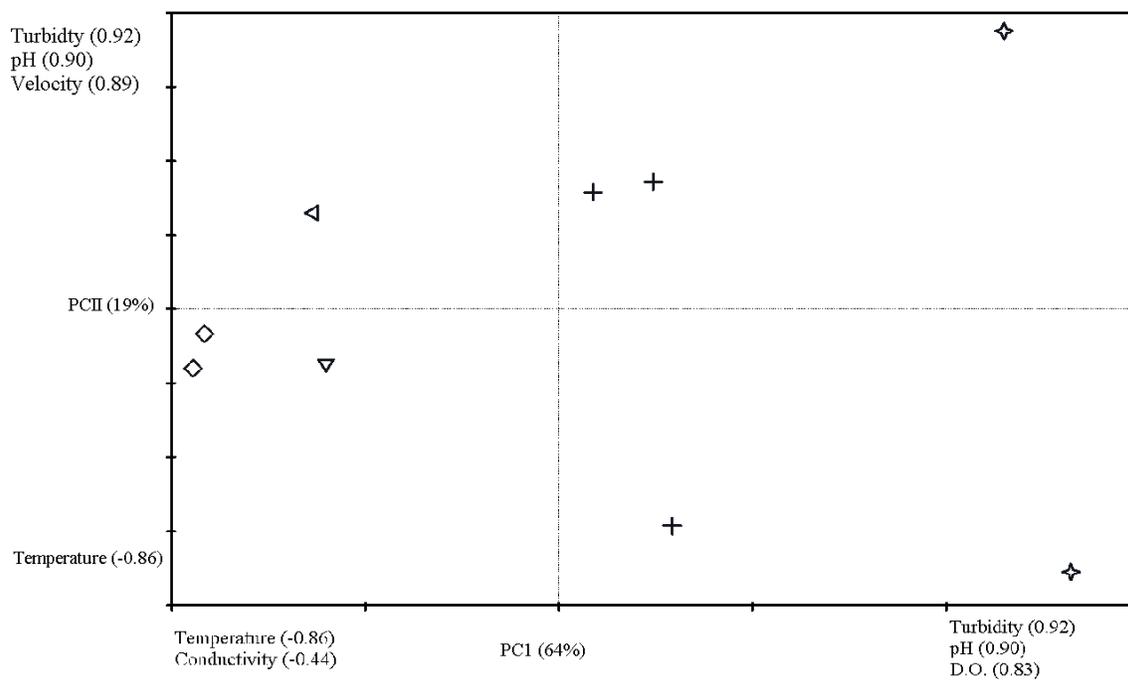


Figure 2. Results of PCA of abiotic variables for study sites: Site 1 (◇), Site 2 (◁), Site 3 (▽), Site 4 (+), and Site 5 (✦) showing biplot scores.

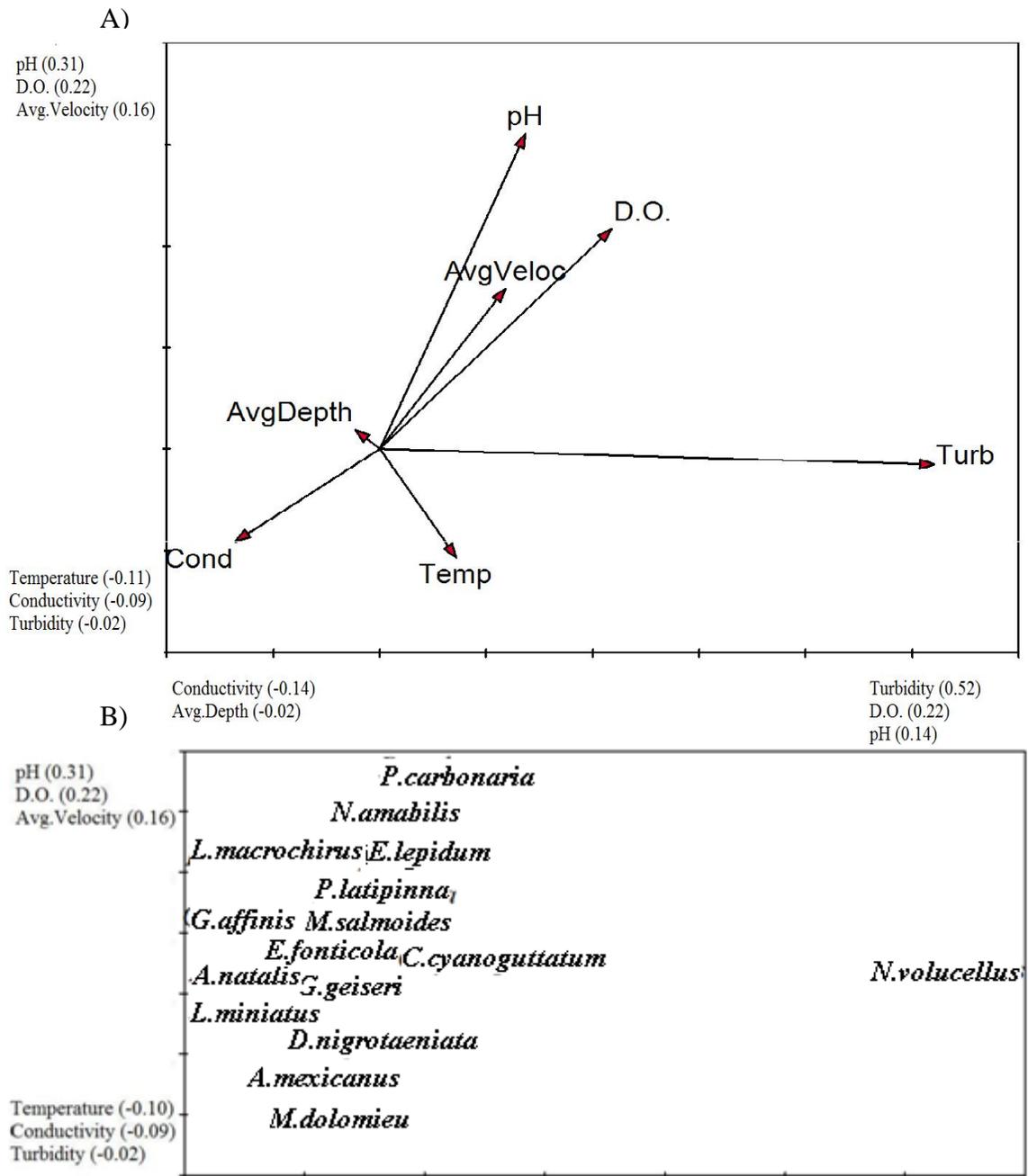


Figure 3. Results of CCA of abiotic characteristics for fish assemblage with loadings (a) and species biplot (b).

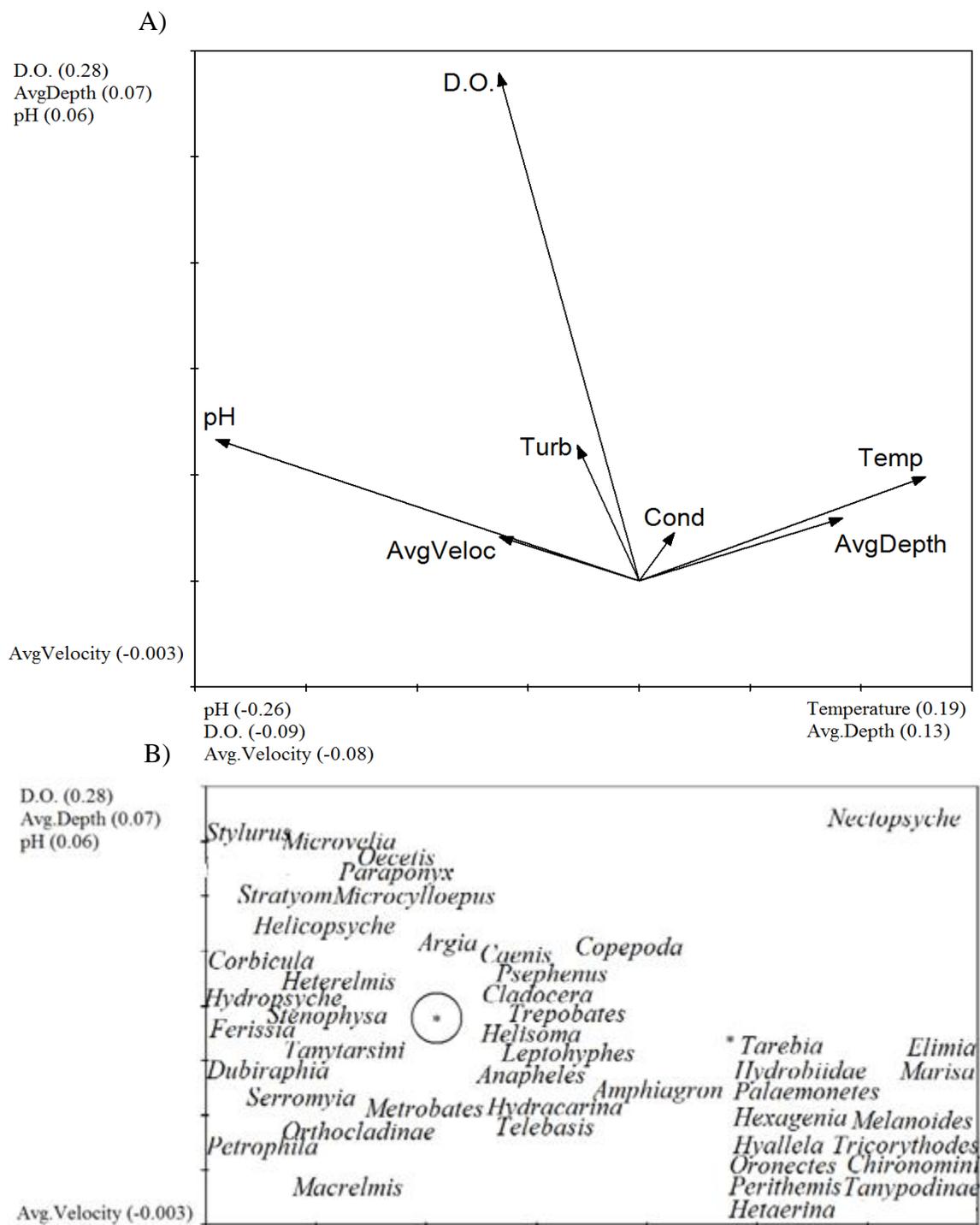
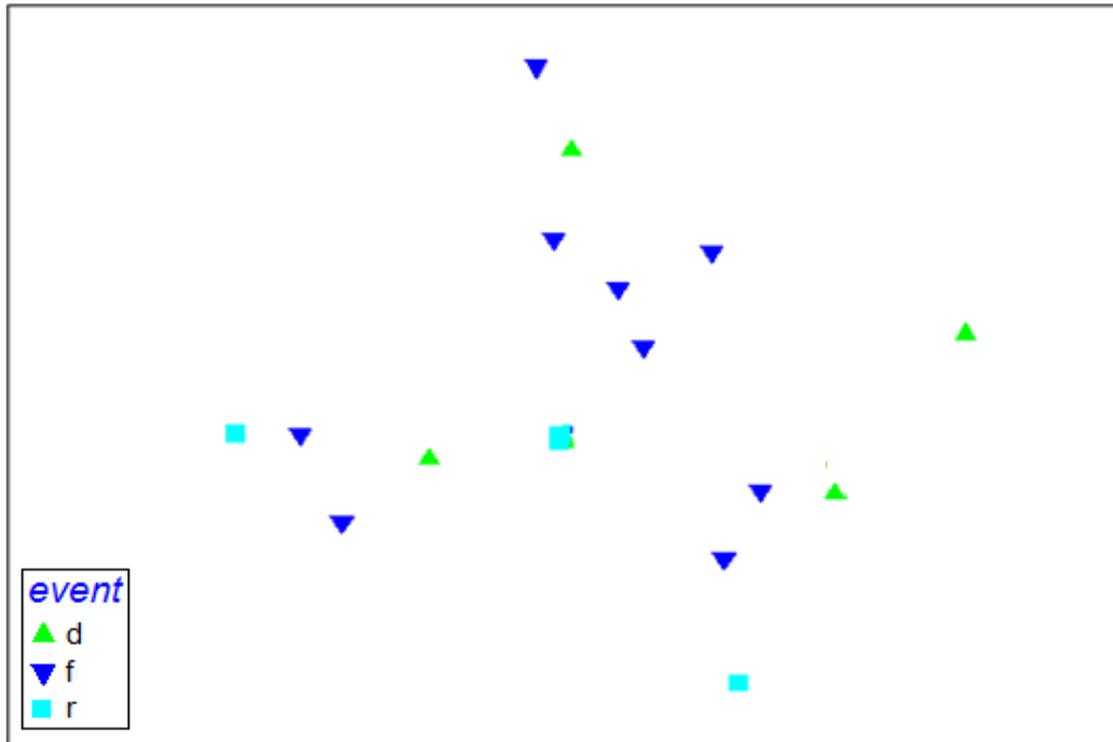


Figure 4. Results of CCA of abiotic characteristics for macroinvertebrate assemblage with loadings (a) and species biplot (b). (*) denotes taxa plotted out near the origin of multivariate space



.Figure 5. Multidimensional scaling (MDS) plot for Comal River sample fish assemblages, with drought, recovery, and post-flood groupings.

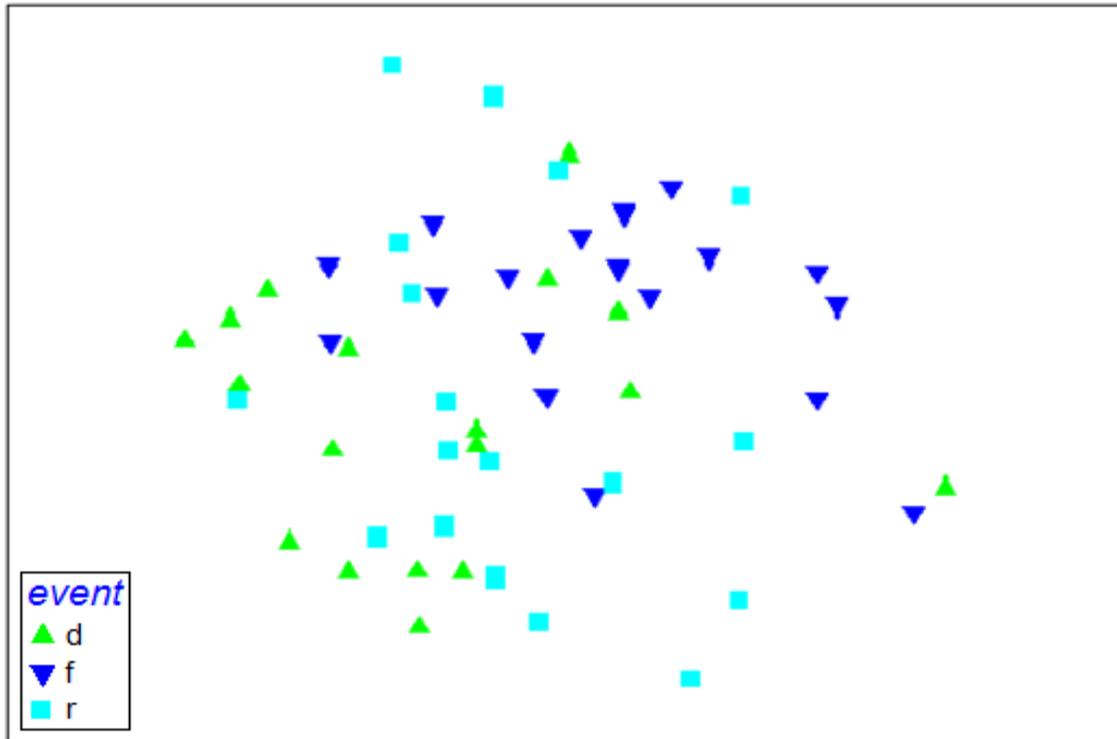


Figure 6. Multidimensional scaling (MDS) plot for Comal River sample macroinvertebrate assemblages, with drought, recovery, and post-flood groupings.

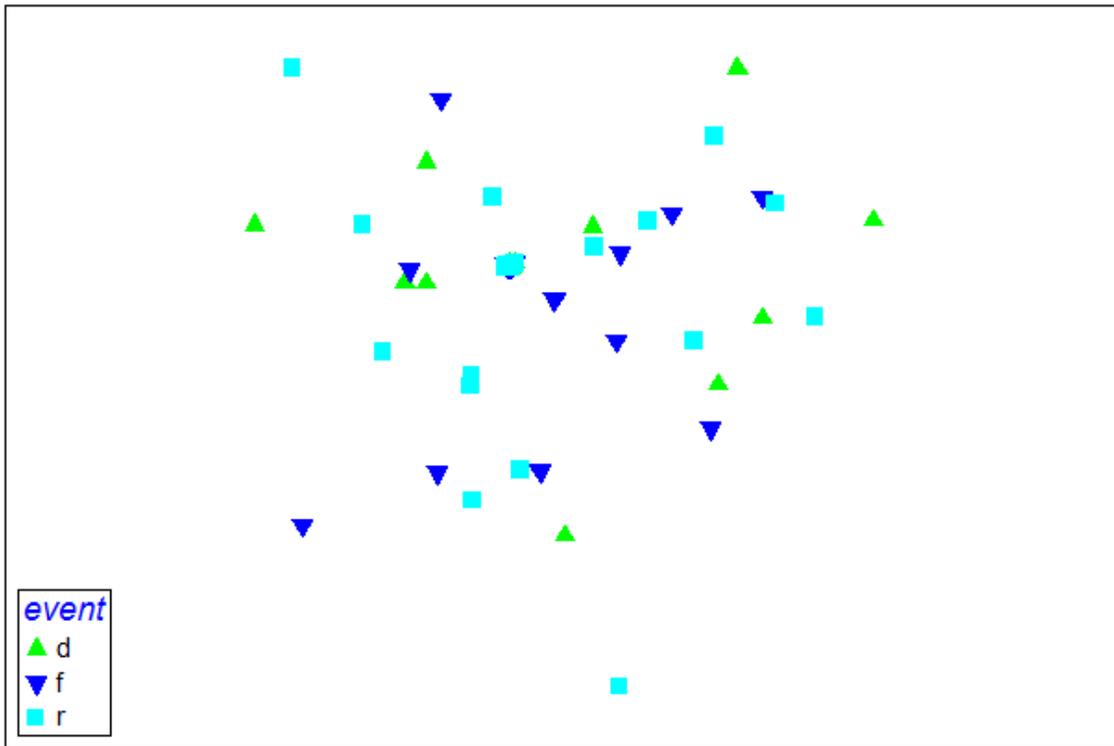


Figure 7. Multidimensional scaling (MDS) plot for Comal River sample diet data, with drought, recovery, and post-flood groupings

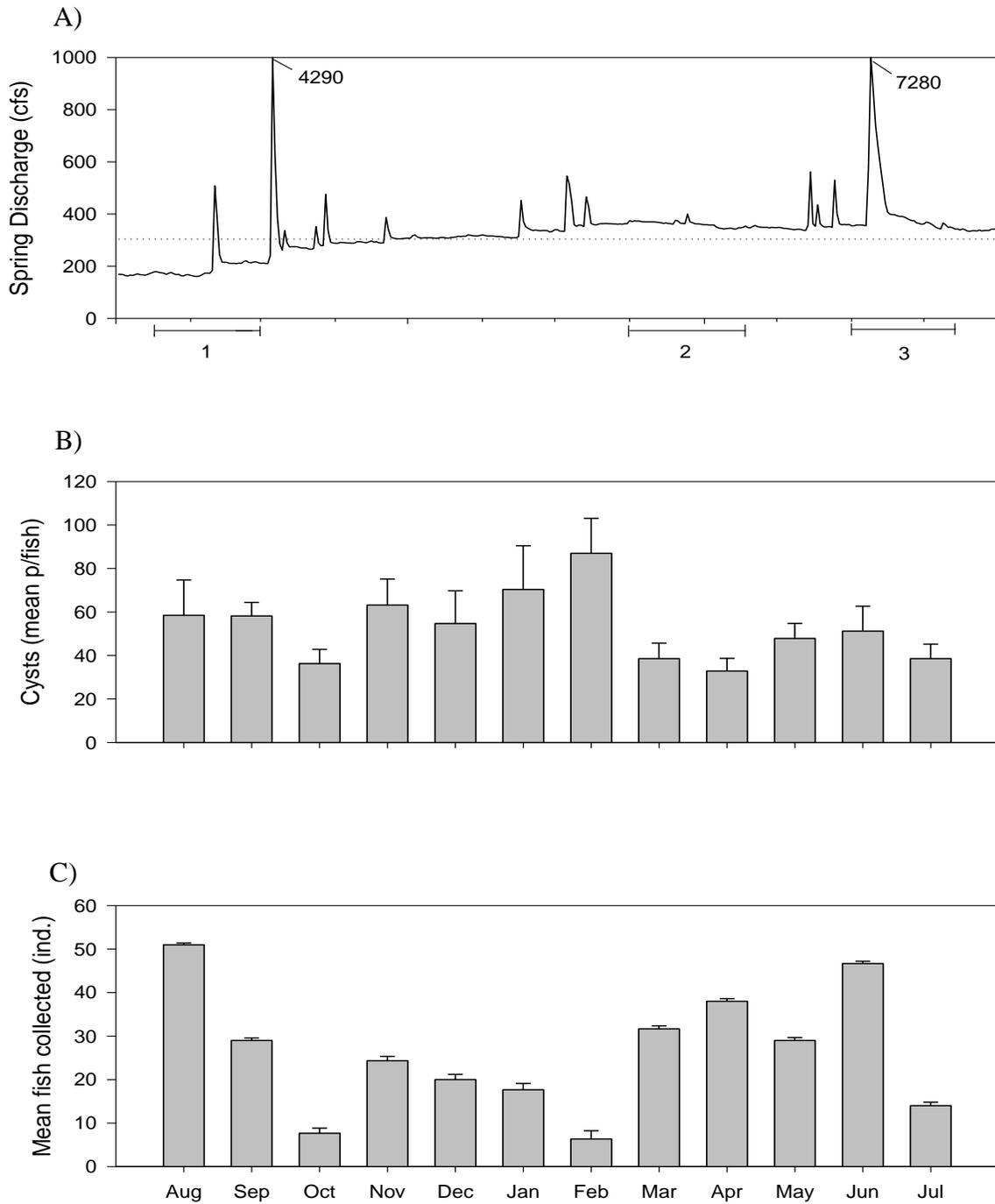


Figure 8. Temporal variation in discharge for 2009-2010 with 1) drought, 2) recovery, and 3) post-flood periods, *Centrocestus formosanus* cyst densities in resident fountain darters, and resident fountain darters captured in the Comal River, Comal County, Texas. A. Current velocity in meters per second. B. Mean (+SE) cyst abundance per fish examined with no significant differences by month ($F_{11, 210} = 1.10$, $P = 0.359$). C. Mean (+SE) fish collected with significant differences by sampling month ($F_{11, 931} = 10.318$, $P < 0.001$).

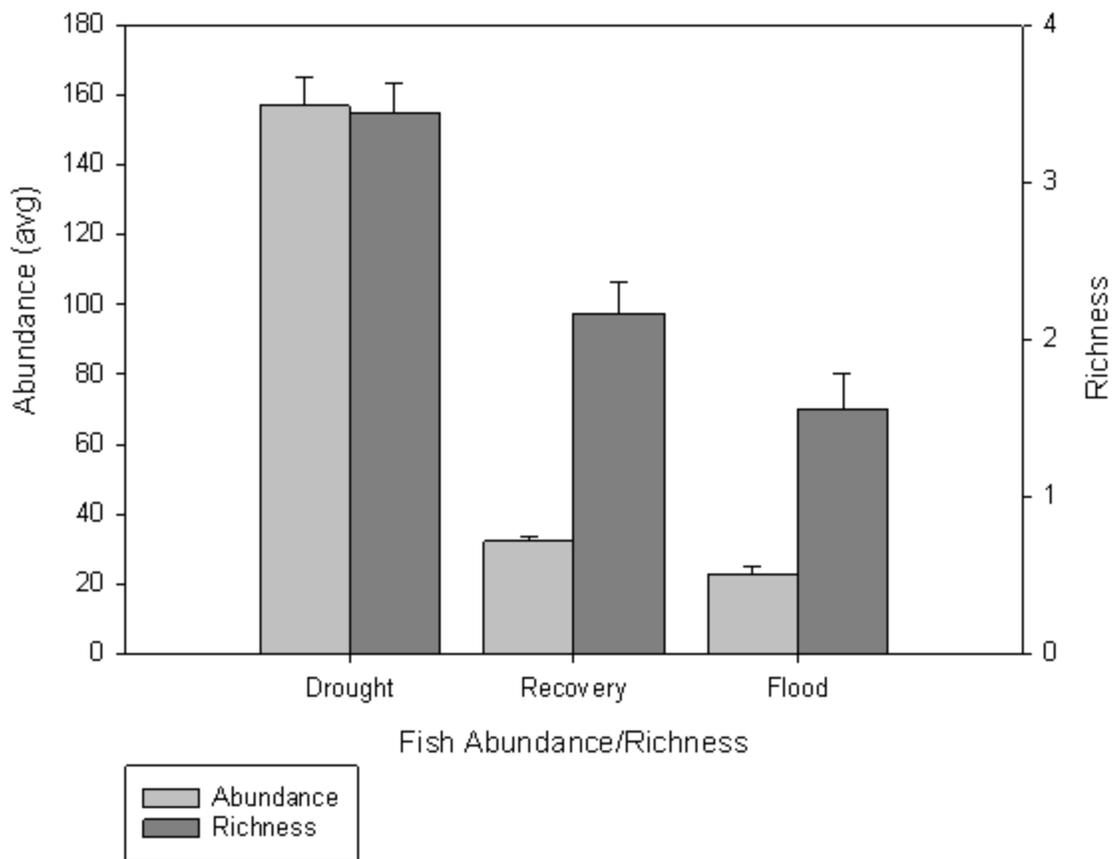


Figure 9. Fish abundance and richness (+SE) in the Comal River categorized by period. Fish abundance ($F_{2,105} = 5.802$, $P < 0.01$) and fish richness ($F_{2,105} = 8.909$, $P < 0.001$) varied significantly by period.

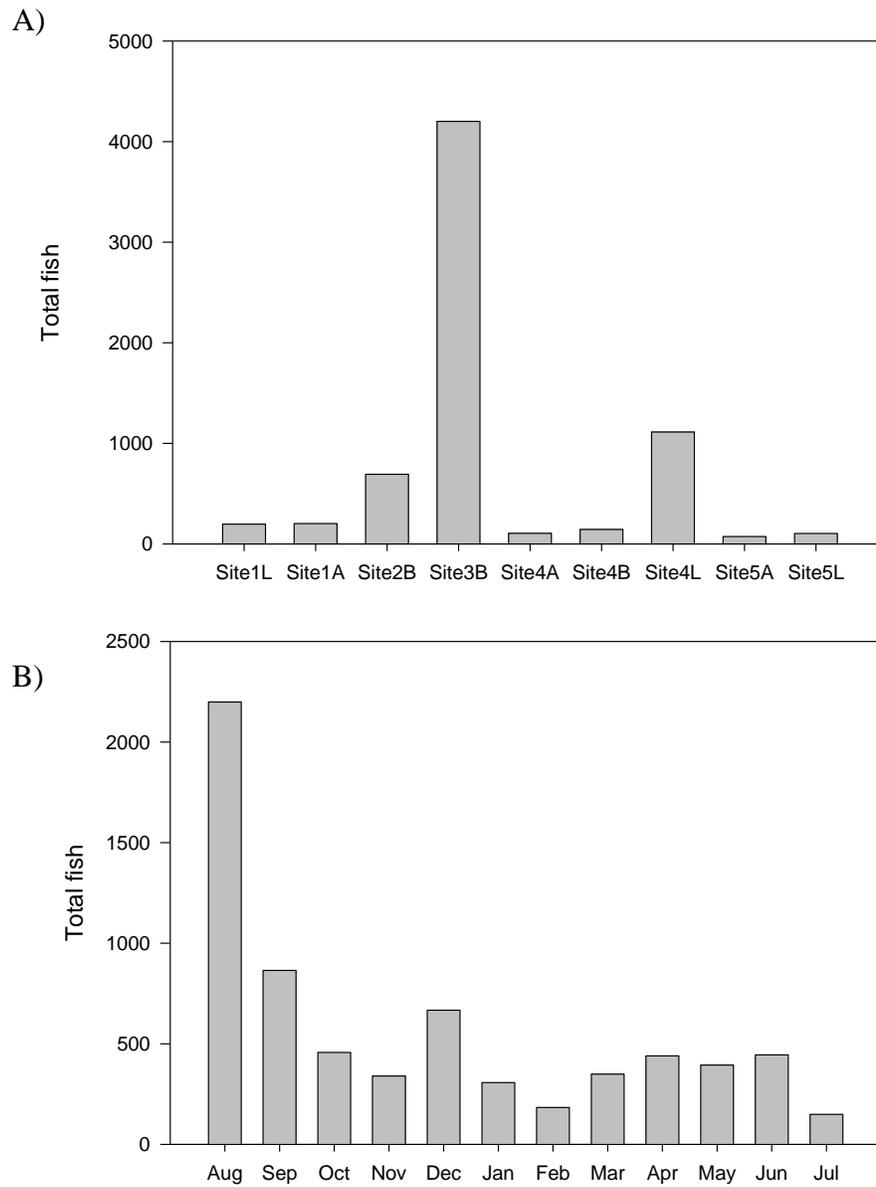


Figure 10. Total fish caught by microhabitat and by month throughout duration of this study. Total fish caught by microhabitat (a) varied significantly among sites ($F_{8,10251} = 54.200$, $P < 0.001$) and among (b) sampling months ($F_{8,10248} = 14.293$, $P < 0.001$).

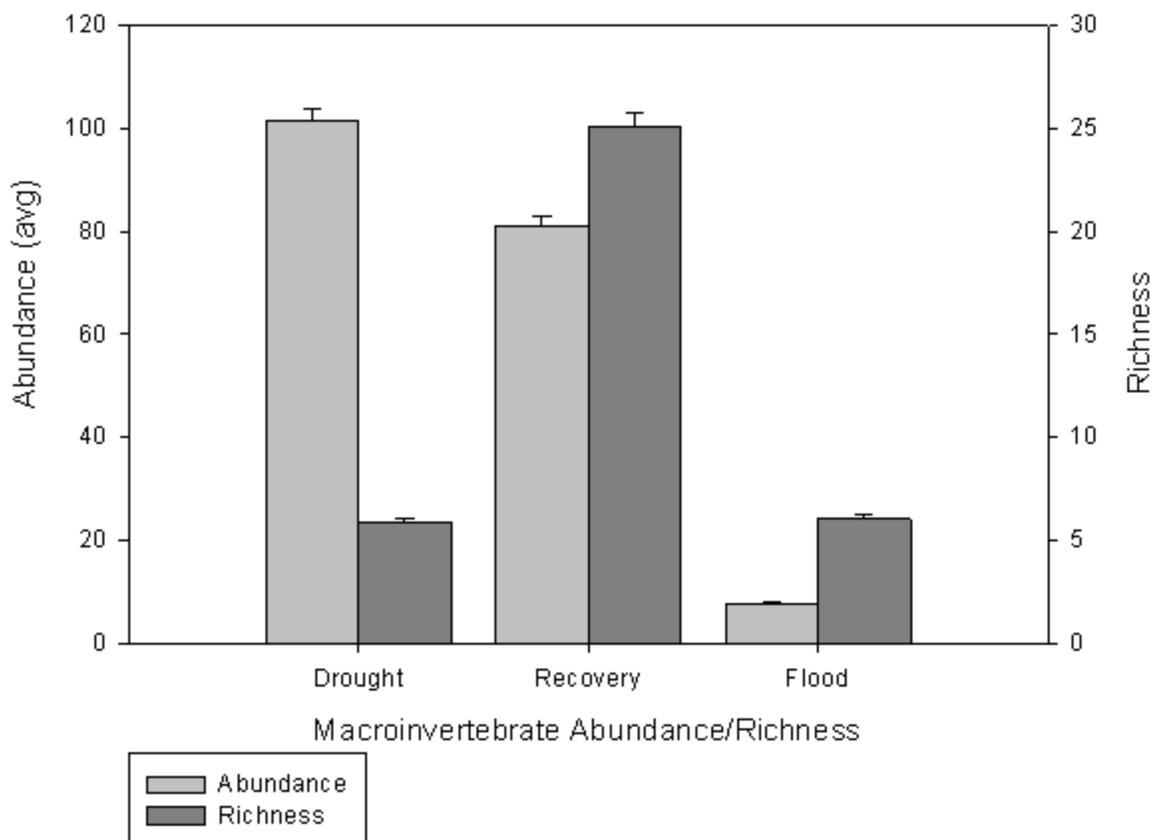


Figure 11. Macroinvertebrate abundance (+SE), and richness in the Comal River categorized by period. Macroinvertebrate abundance ($F_{2,104} = 10.635$, $P < 0.001$) and richness ($F_{2,105} = 4.481$, $P < 0.05$) varied among periods.

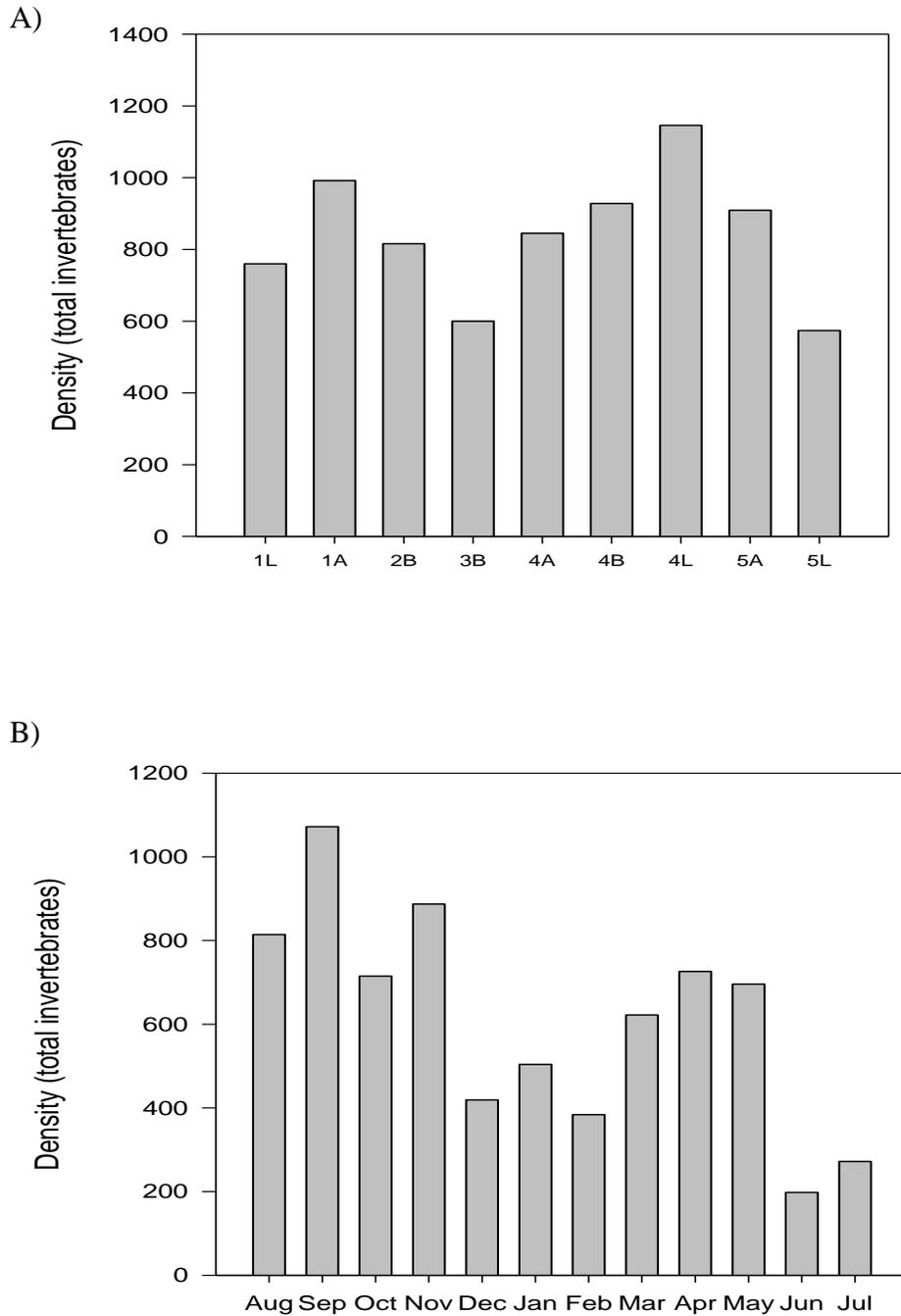


Figure 12. Macroinvertebrate densities collected during benthic sampling by (a) site and (b) month. Total macroinvertebrates collected varied significantly with respect to sampling month ($F_{11,5604} = 2.279$, $P < 0.01$). No significant differences among sites ($F_{8,5607} = 1.617$, $P = 0.113$) were found with respect to macroinvertebrate densities.

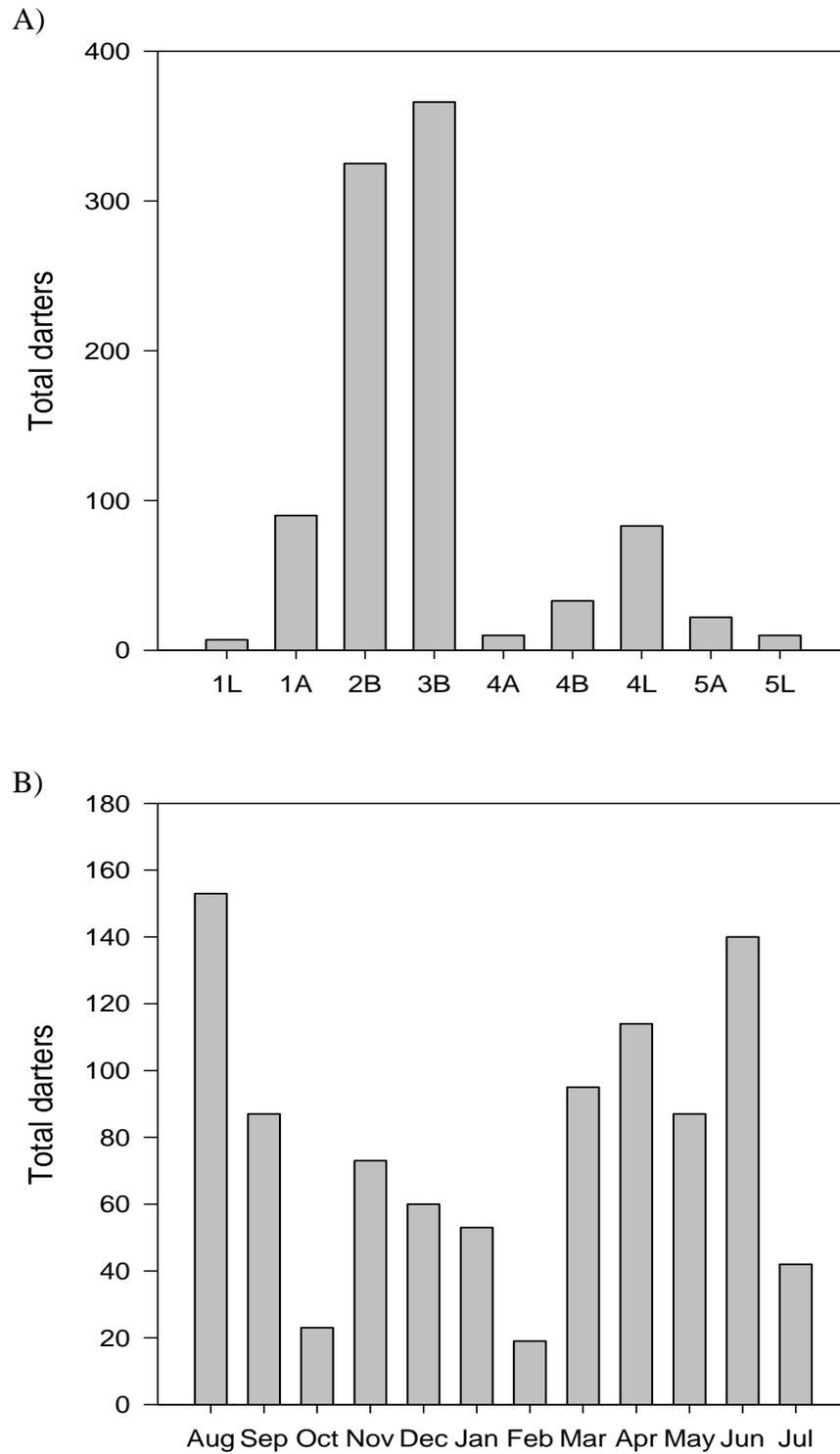


Figure 13. Total fountain darters collected by microhabitat and sampling month throughout study period. Total darters collected by (a) microhabitat ($F_{8,934} = 43.230$, $P < 0.001$) and by (b) sampling month ($F_{11,931} = 10.318$, $P < 0.001$), varied significantly during study.

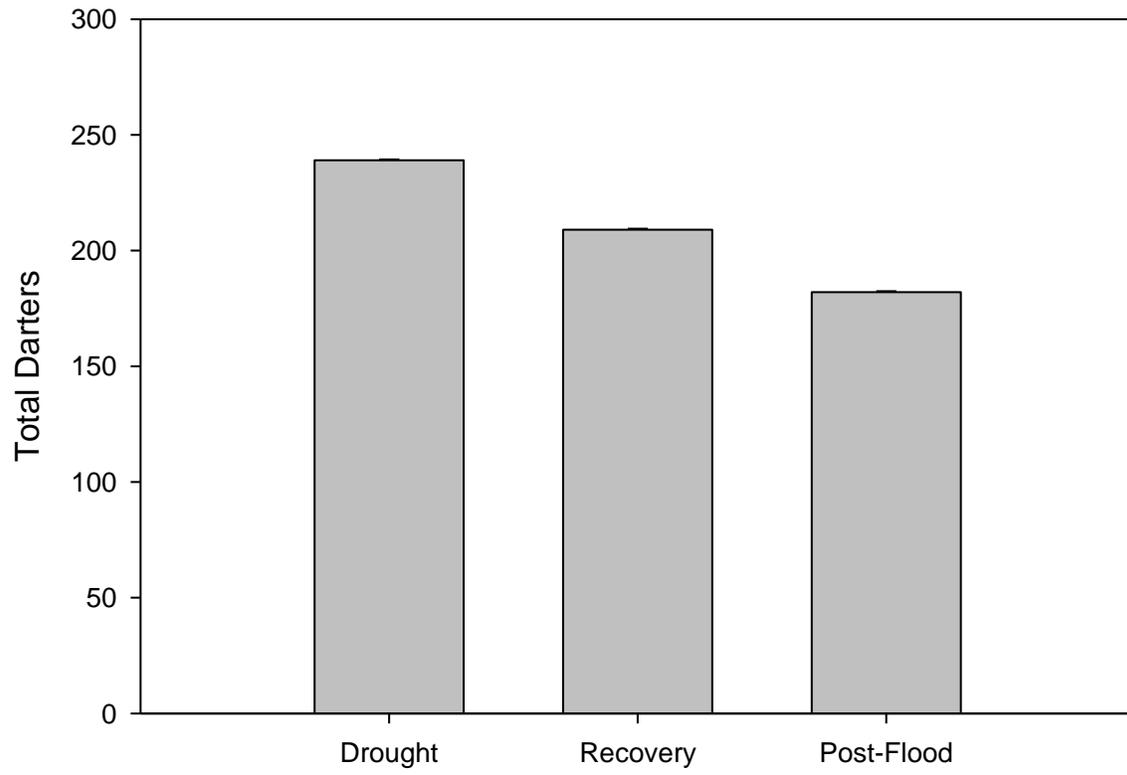


Figure 14. Fountain darter densities by study period. Total fountain darters varied significantly among study periods ($F_{2,628} = 42.819$, $P < 0.001$).

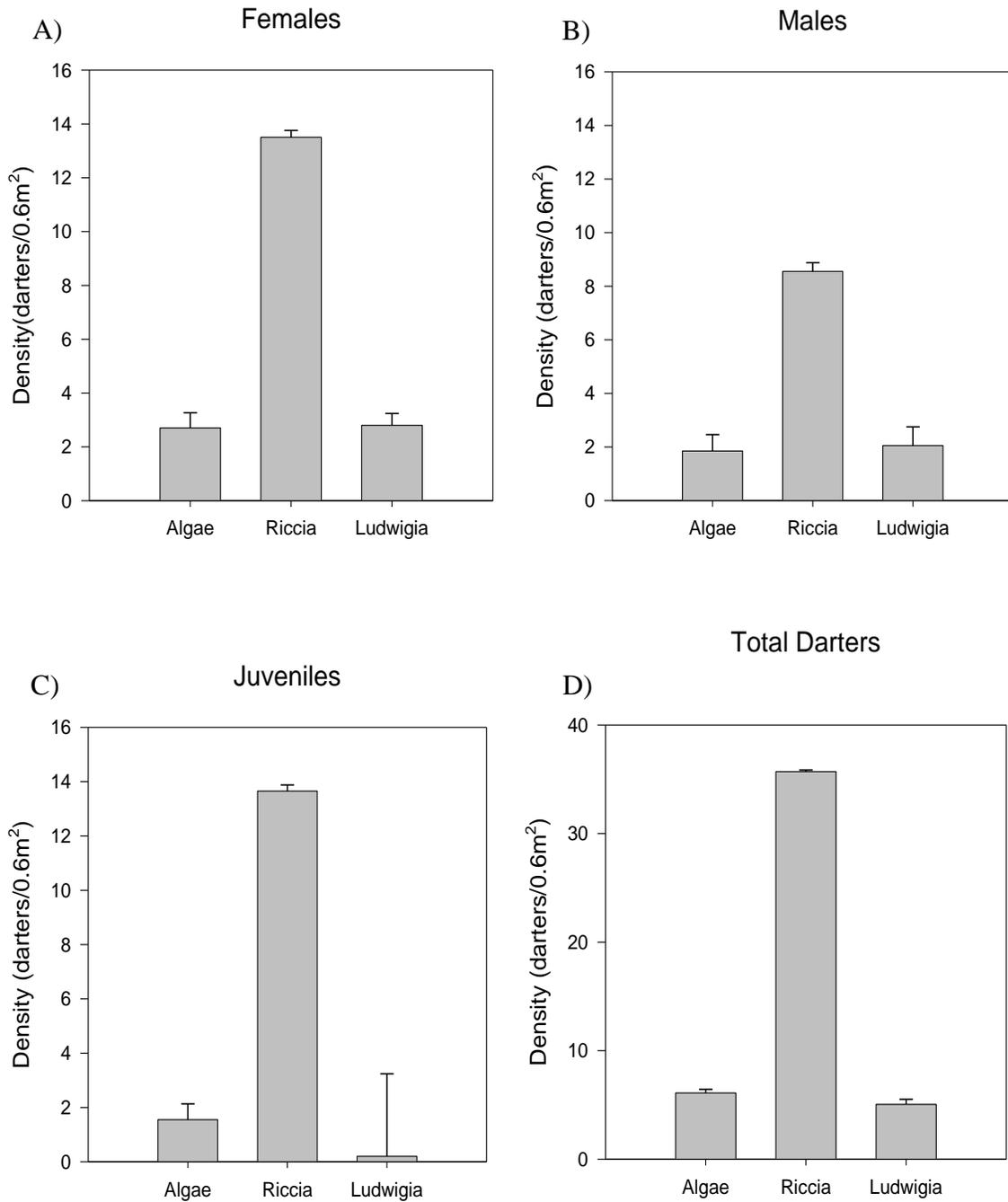


Figure 15. Fountain darter (+SE) collected per square meter by microhabitat. (a) Females ($F_{2,387} = 14.689$, $P < 0.001$), (b) males ($F_{2,246} = 6.692$, $P < 0.01$), and (d) total darters ($F_{2,944} = 37.833$, $P < 0.001$) varied significantly among microhabitats. (c) Juveniles ($F_{2,305} = 0.091$, $P = 0.913$) showed no significance with respect to microhabitat selection.

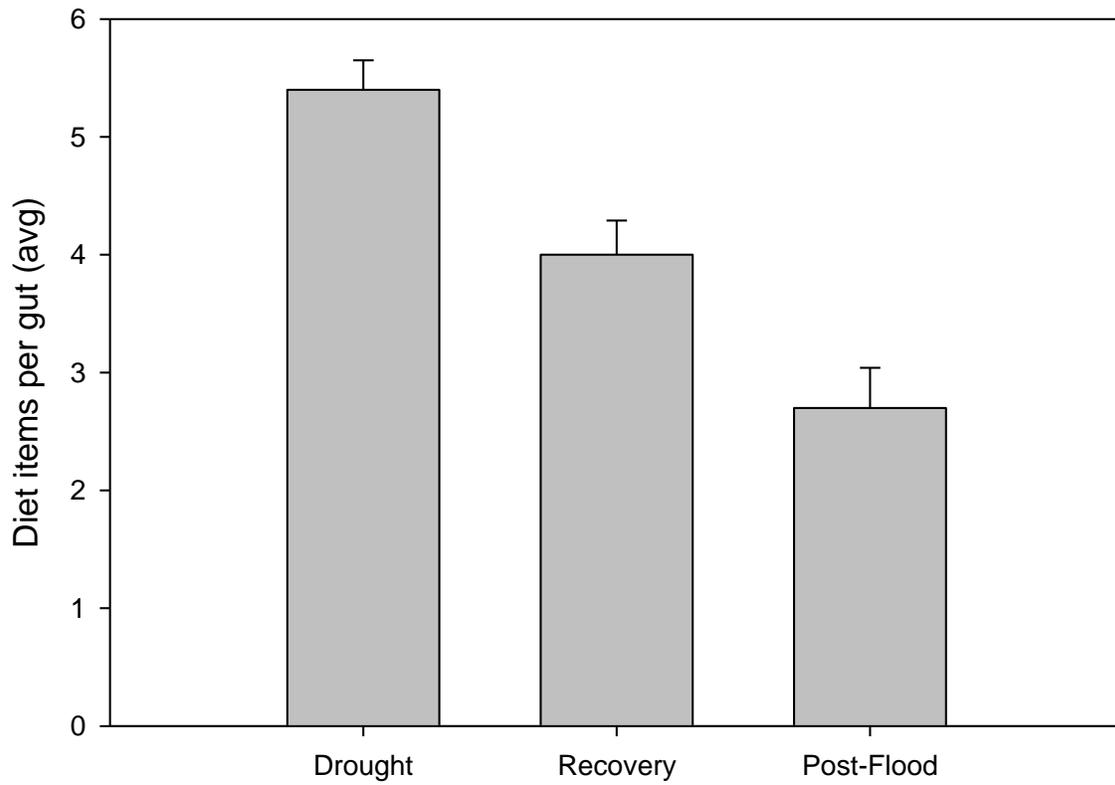


Figure 16. Fountain darter diet items per digestive tract. Average diet items per digestive tract varied significantly among study periods ($F_{2,264} = 5.575$, $P < 0.01$).

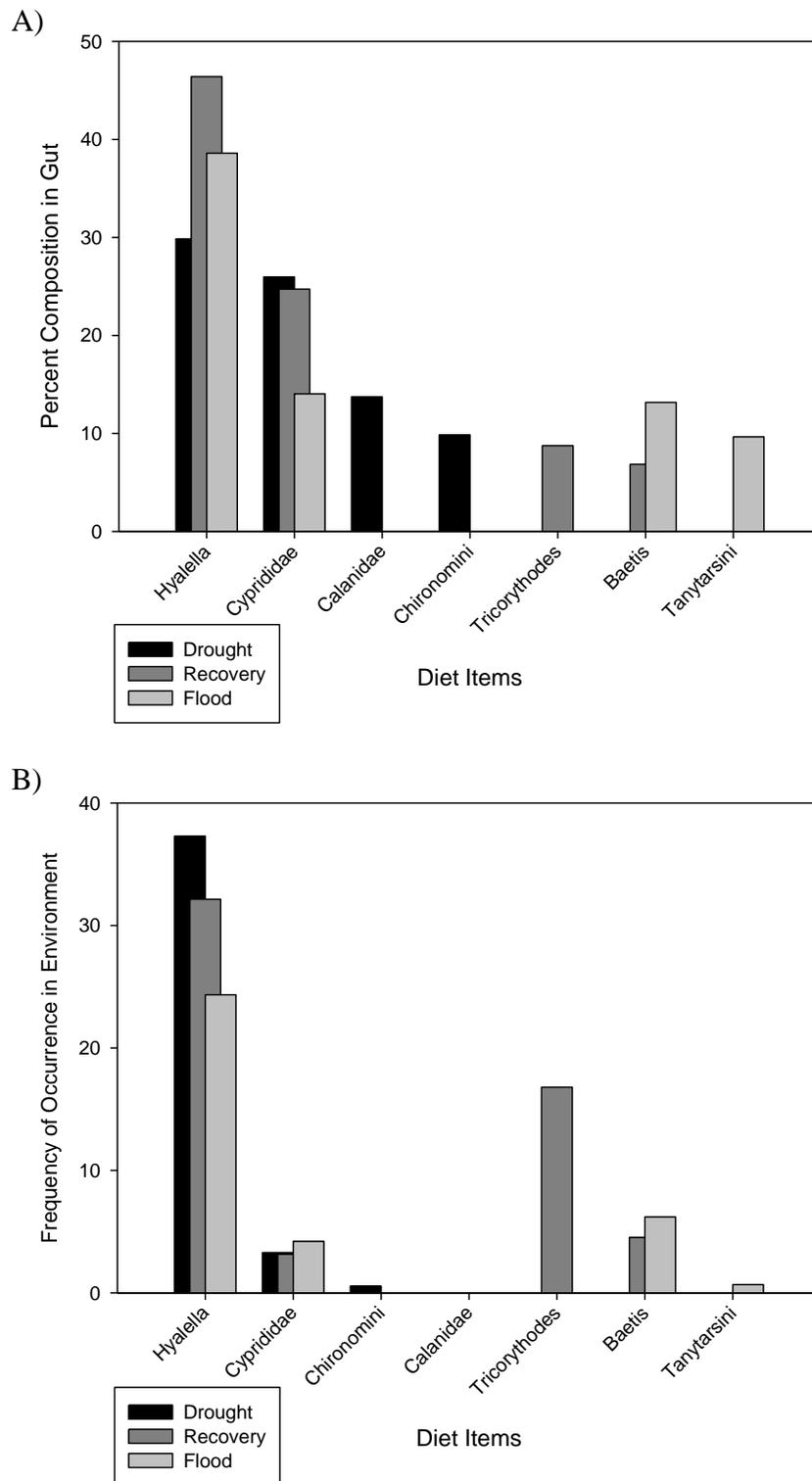


Figure 17. Percent composition in gut (a) and frequency of occurrence in the environment (b) of macroinvertebrates in the Comal River.

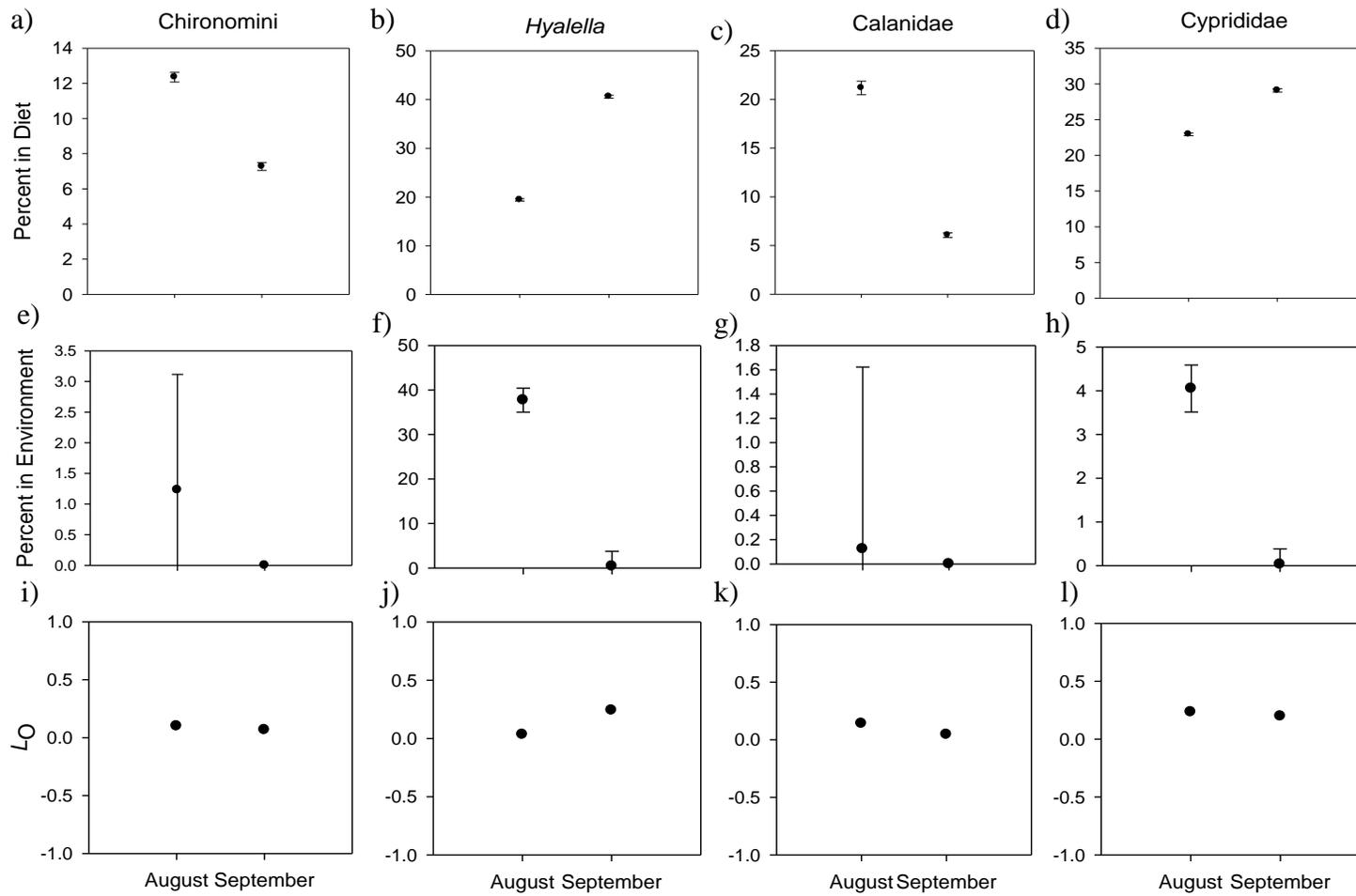


Figure 18. Percent composition in the diet (a-d), environment (e-h), and linear electivity (i-l) of Chironomini, *Hyalella*, Calanidae, and Cyprididae during drought period.

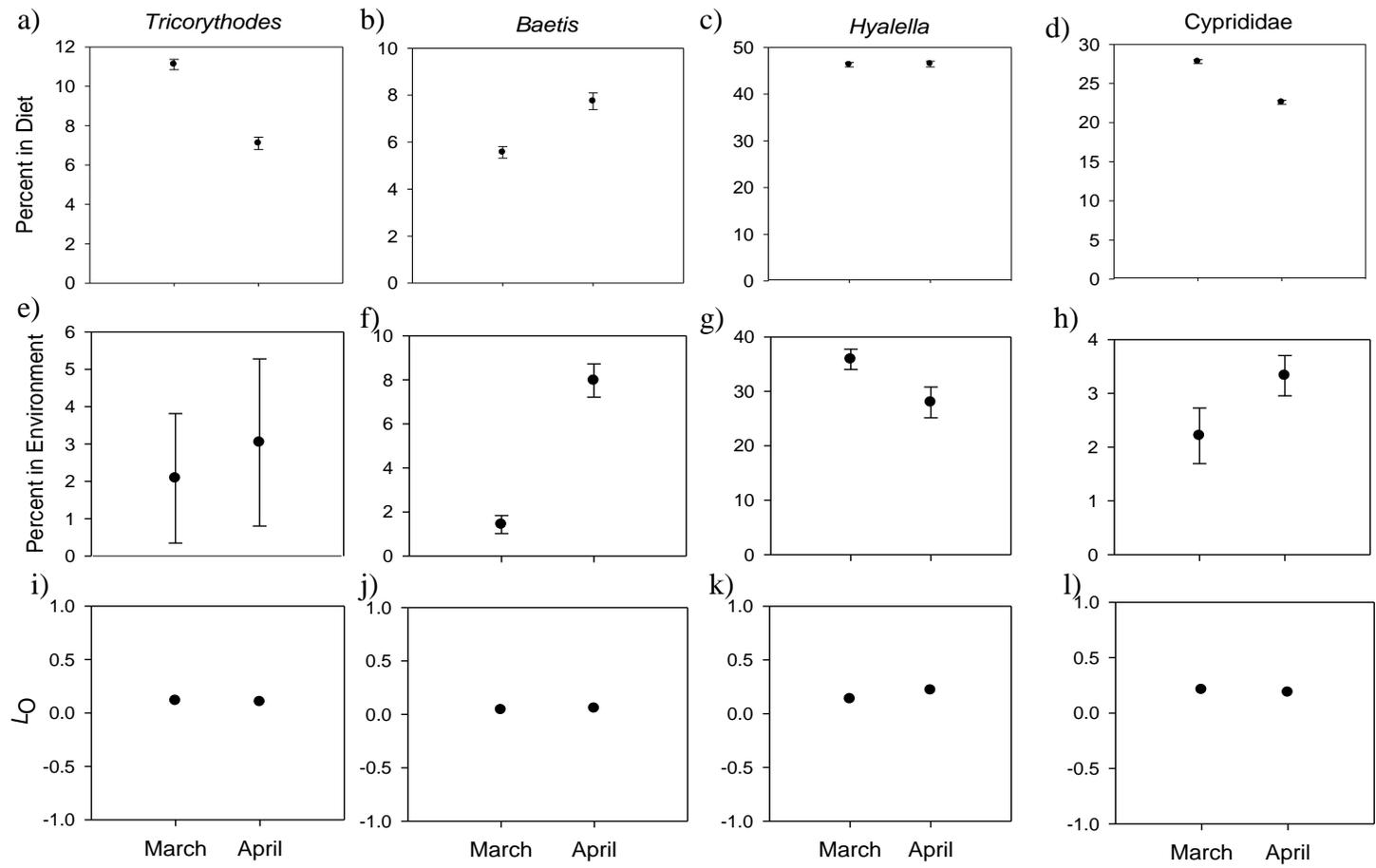


Figure 19. Percent composition in the diet (a-d), environment (e-h), and linear electivity (i-l) of *Tricorythodes*, *Baetis*, *Hyalella*, and *Cyprididae* during recovery period.

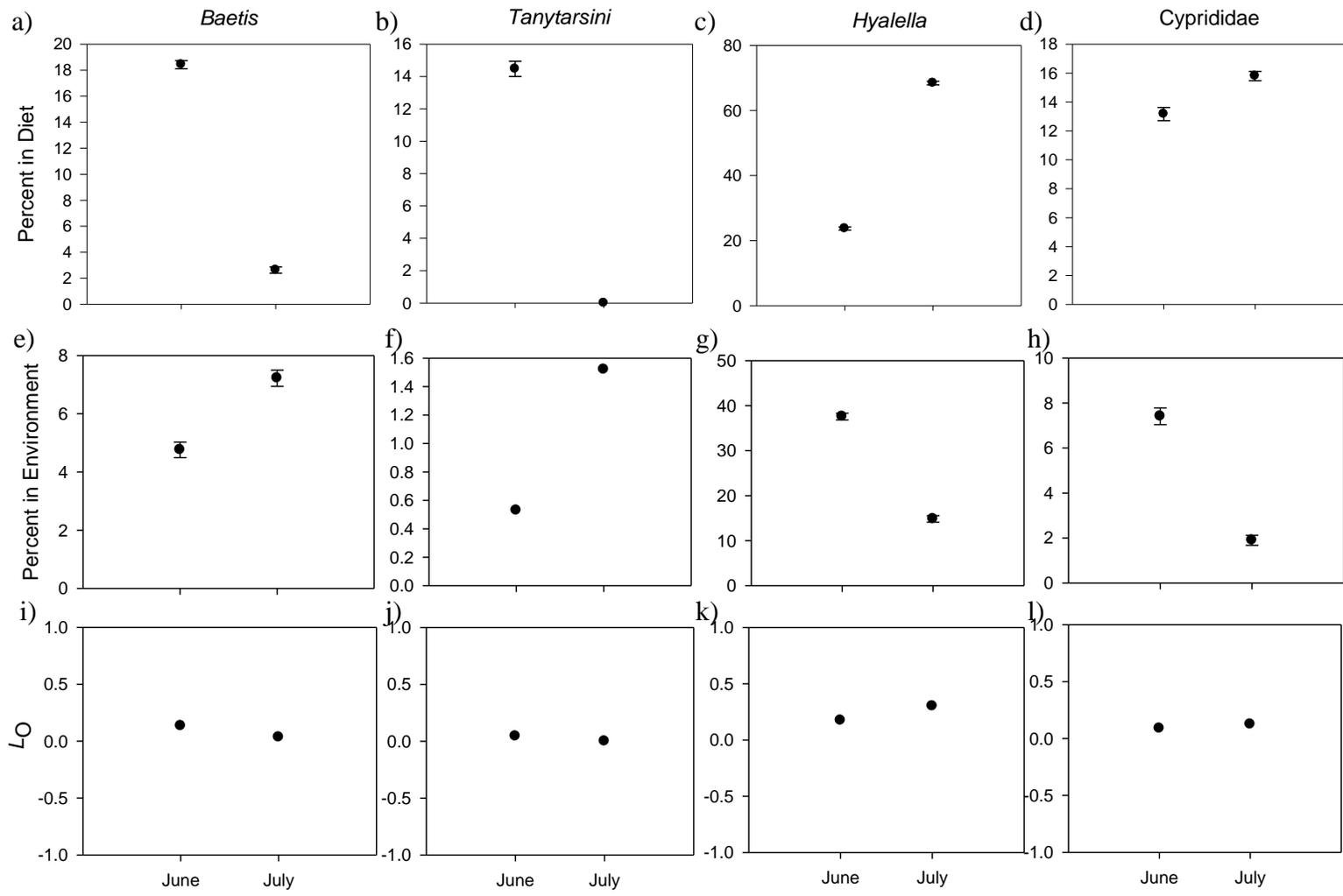


Figure 20. Percent composition in the diet (a-d), environment (e-h), and linear electivity (i-l) of *Baetis*, *Tanytarsini*, *Hyalella*, and *Cyprididae* during post-flood period

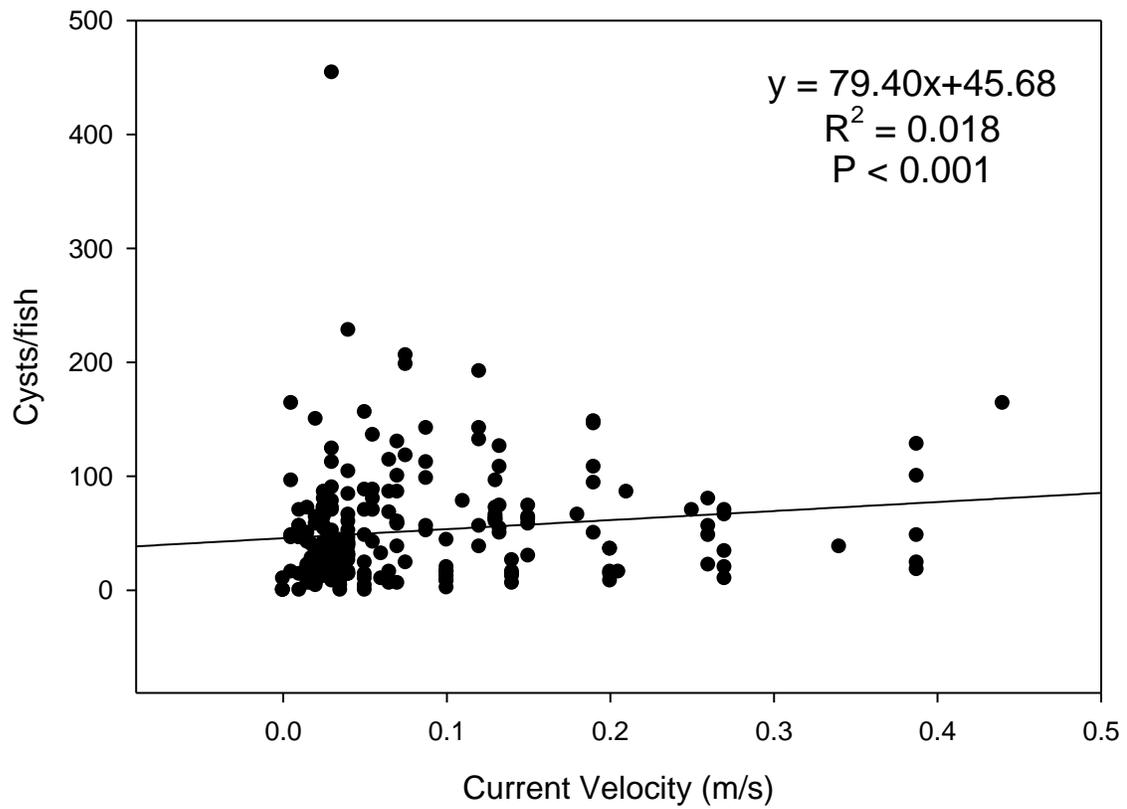


Figure 21. The effects of current velocity (m/s) on *Centrocestus formosanus* abundance.
A. Current velocity regressed against densities in resident fountain darters.

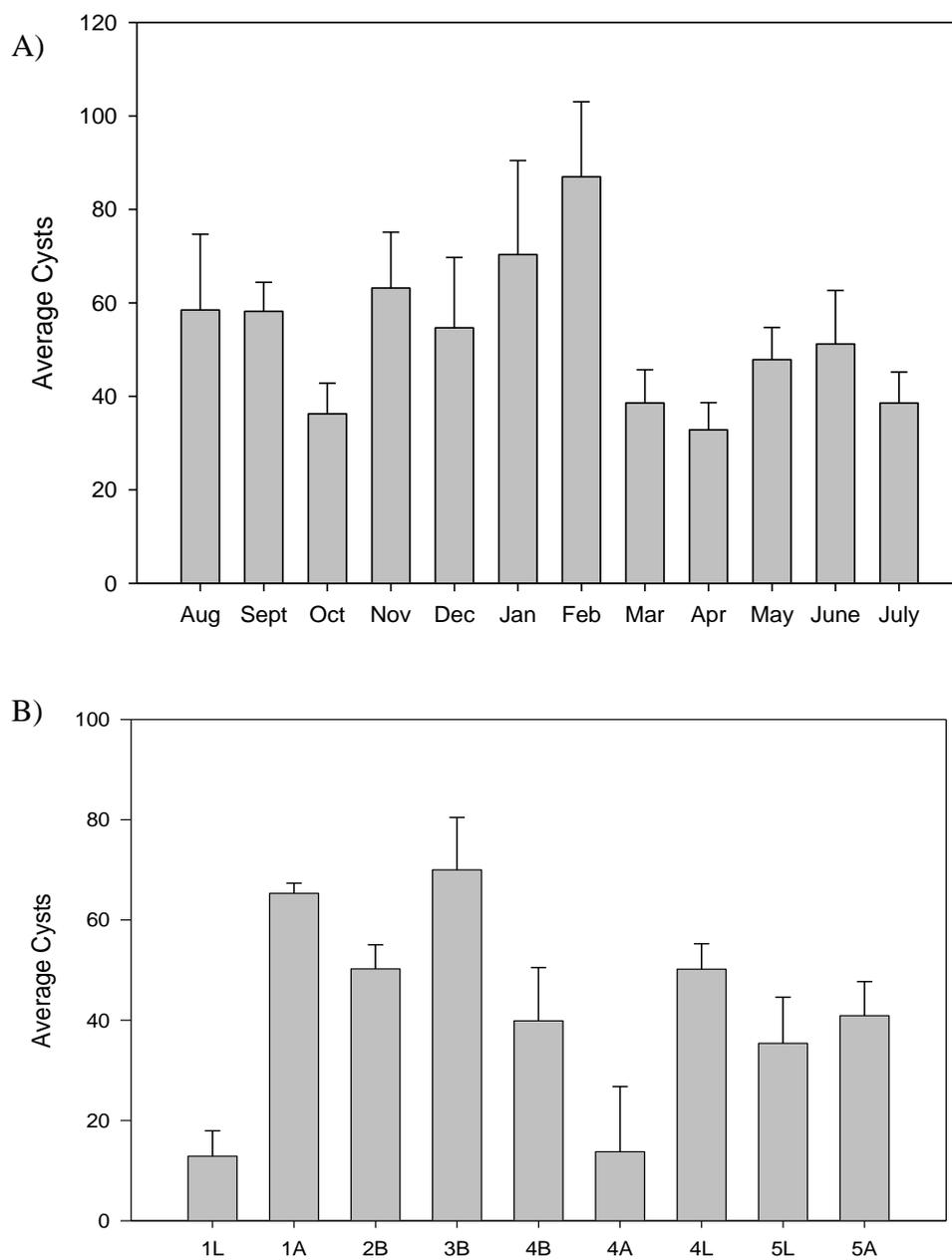


Figure 22. *Centrocestus formosanus* densities depicted by sampling month and by site. (A) Total parasite densities did not vary significantly by sampling month ($F_{11,210} = 1.104$, $P = 0.359$), however did vary significantly by (B) site ($F_{8,213} = 2.952$, $P < 0.01$).

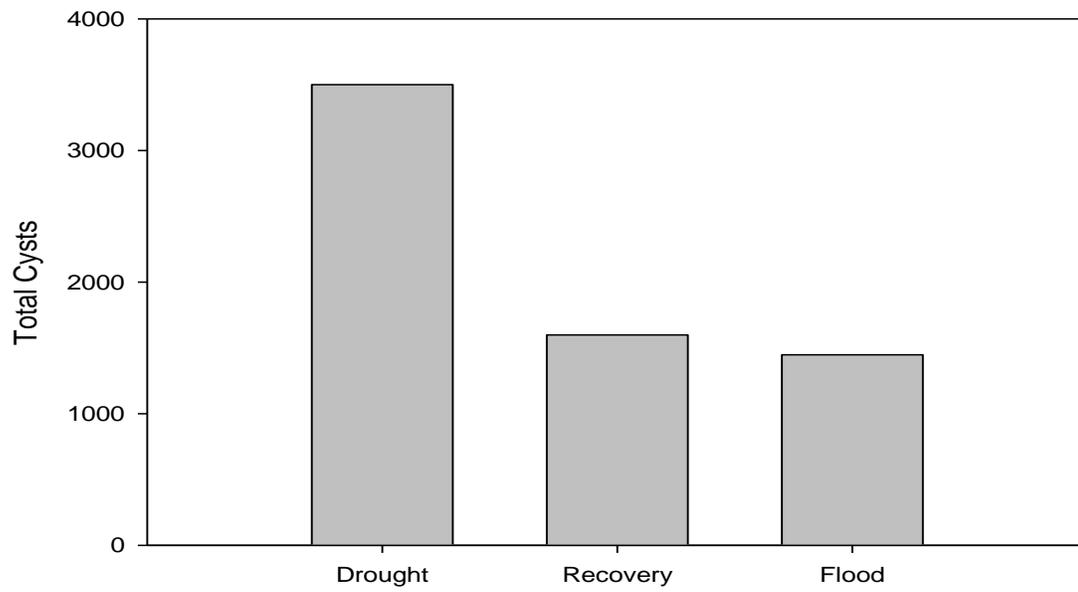


Figure 23. *Centrocestus formosanus* densities depicted by period. Densities of the parasite varied significantly by period ($F_{2,133} = 3.305$, $P < 0.05$).

APPENDIX

Table A - 1 . Aquatic invertebrates collected from benthic sampling during this study.

Order/sub-order	Family	Sub – Family	Genus
Ephemeroptera	Baetidae		<i>Baetis</i>
Ephemeroptera	“		<i>Callibaetis</i>
Ephemeroptera	“		<i>Fallceon</i>
Ephemeroptera	Caenidae		<i>Caenis</i>
Ephemeroptera	Ephemeridae		<i>Hexagenia</i>
Ephemeroptera	Leptohyphidae		<i>Leptohyphes</i>
Ephemeroptera	“		<i>Tricorythodes</i>
Odonata/Anisoptera	Gomphidae		<i>Stylurus</i>
Odonata/Anisoptera	Libellulidae		<i>Perithemis</i>
Odonata/Zygoptera	Calopterygidae		<i>Hetaerina</i>
Odonata/Zygoptera	Coenagrionidae		<i>Amphiagron</i>
Odonata/Zygoptera	“		<i>Argia</i>
Odonata/Zygoptera	“		<i>Telebasis</i>
Trichoptera	Helicopsychidae		<i>Helicopsyche</i>
Trichoptera	Hydropsychidae		<i>Hydropsyche</i>
Trichoptera	“		<i>Nectopsyche</i>
Trichoptera	Leptoceridae		<i>Oecetis</i>
Lepidoptera	Crambidae		<i>Paraponyx</i>
Lepidoptera	“		<i>Petrophila</i>
Hemiptera	Gerridae		<i>Metrobates</i>
Hemiptera	“		<i>Trepobates</i>
Hemiptera	Veliidae		<i>Microvelia</i>
Coleoptera	Elmidae		<i>Dubiraphia</i>
Coleoptera	“		<i>Heterelmis</i>
Coleoptera	“		<i>Macrelmis</i>
Coleoptera	“		<i>Microcylloepus</i>
Coleoptera	Psephenidae		<i>Psephenus</i>
Diptera	Ceratopogonidae		<i>Serromyia</i>
Diptera	Chironomidae	Chironomini	
Diptera	“	Orthoclaadiinae	
Diptera	“	Tanypodinae	
Diptera	“	Tanytarsini	
Diptera	Culicidae		<i>Anopheles</i>
Amphipoda	Hyaellidae		<i>Hyaella</i>
Decapoda	Cambaridae		<i>Oronectes</i>
Decapoda	Palaemonidae		<i>Palaemonetes</i>
Limnophila	Physidae		<i>Stenophysa</i>
Limnophila	Planorbidae		<i>Helisoma</i>
Limnophila	“		<i>Ferissia</i>
Caenogastropoda	Ampullariidae		<i>Marisa</i>

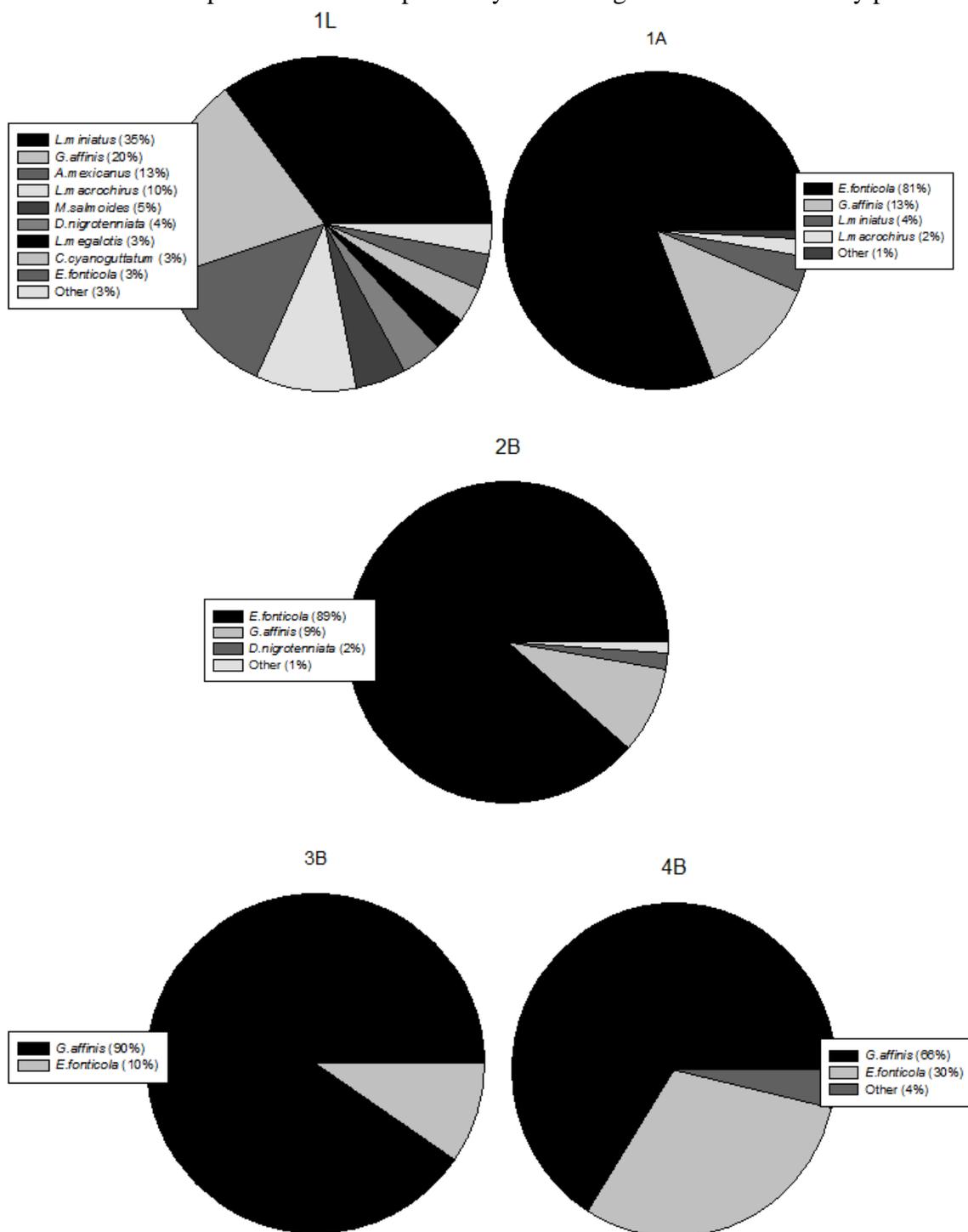
Table a-1 Continued

Order/sub-order	Family	Sub – Family	Genus
Megagastropoda	Hydrobiidae		
Megagastropoda	Pilidae		
Megagastropoda	Pleuroceridae		<i>Elimia</i>
Megagastropoda	Thiaridae		<i>Melanoides</i>
Megagastropoda	“		<i>Tarebia</i>
Veneroida	Corbiculidae		<i>Corbicula</i>
Cladocera	Daphniidae		<i>Daphnia</i>
Calanoida			
Ostracoda	Cyprididae		
Acariformes		Hydracarina	

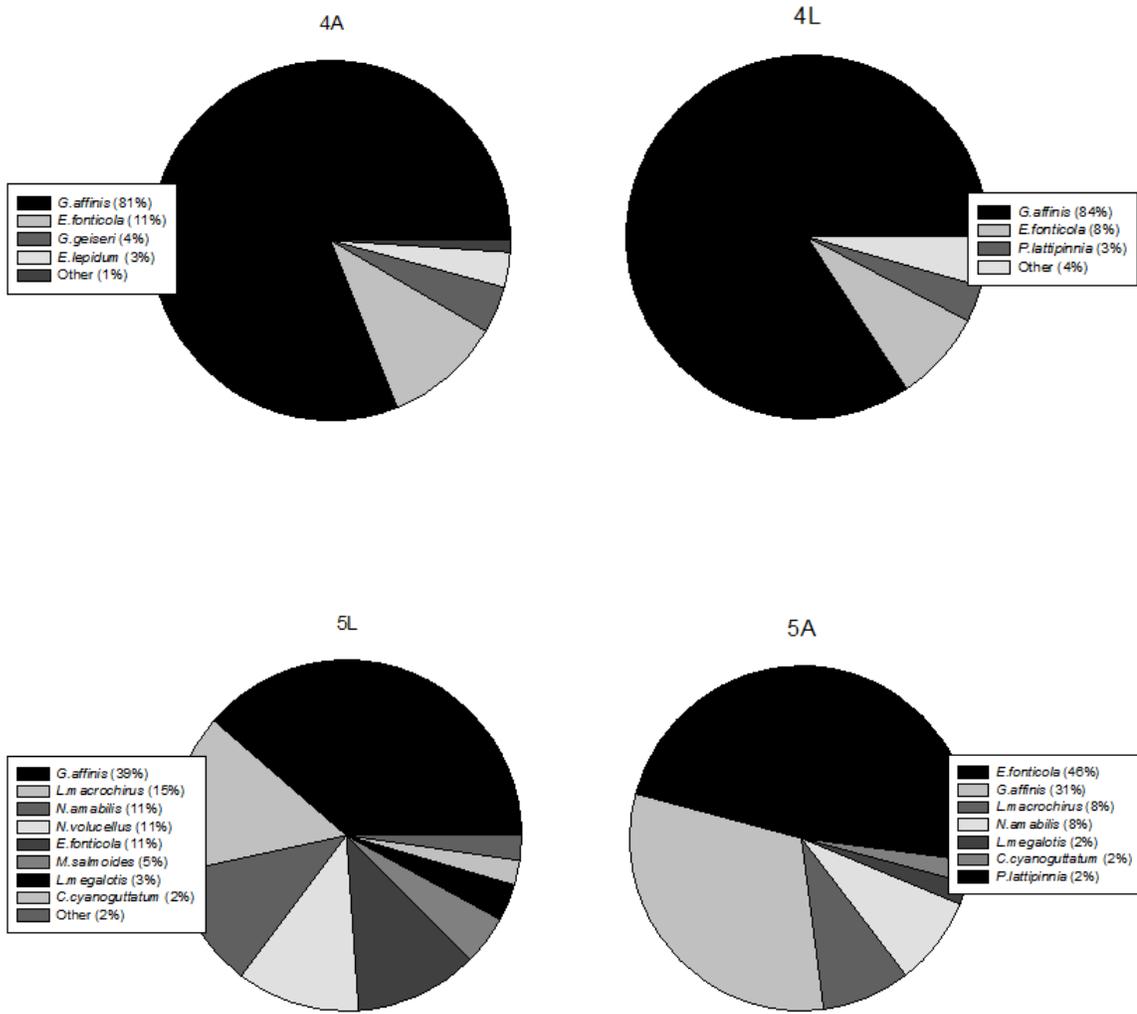
Table A - 2 Fish collected during this study.

Order/sub-order	Family	Sub – Family	Genus
Characiformes	Characidae		<i>Astyanax mexicanus</i>
Cypriniformes	Cyprinidae		<i>Dionda nigrotaeniata</i>
Cypriniformes			<i>Notropis amabilis</i>
Cypriniformes			<i>Notropis volucellus</i>
Cyprinodontiformes	Poeciliidae		<i>Gambusia affinis</i>
Cyprinodontiformes			<i>Gambusia geiseri</i>
Cyprinodontiformes			<i>Poecilia latipinna</i>
Perciformes	Centrarchidae		<i>Lepomis macrochirus</i>
Perciformes			<i>Lepomis megalotis</i>
Perciformes			<i>Lepomis miniatus</i>
Perciformes			<i>Micropterus salmoides</i>
Perciformes	Cichlidae		<i>Cichlasoma cyanoguttatum</i>
Perciformes	Percidae		<i>Etheostoma fonticola</i>
Perciformes			<i>Etheostoma lepidum</i>
Perciformes			<i>Percina carbonaria</i>
Siluriformes	Ictaluridae		<i>Ameiurus natalis</i>

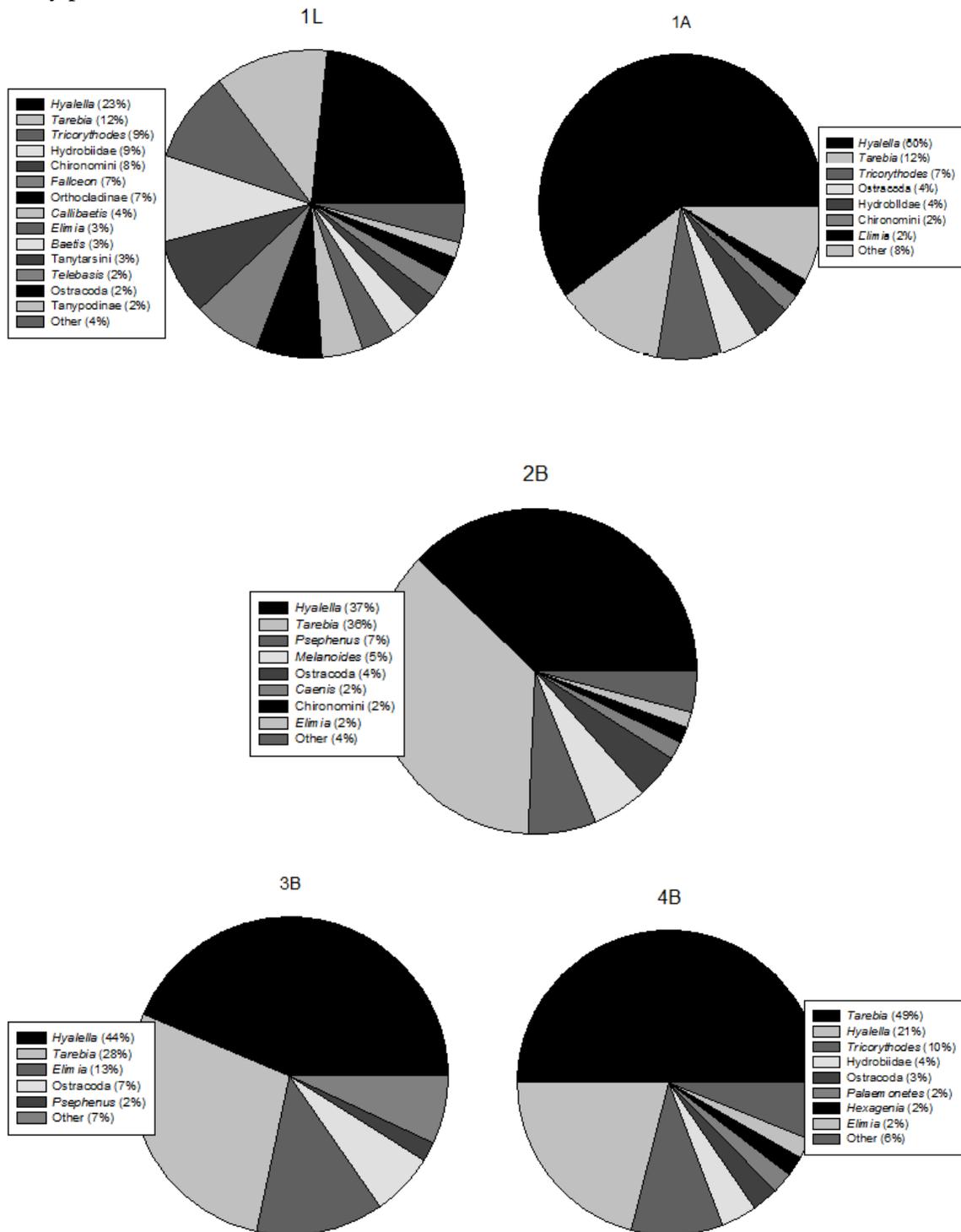
A - 3. Percent composition of fish captured by site throughout duration of study period.



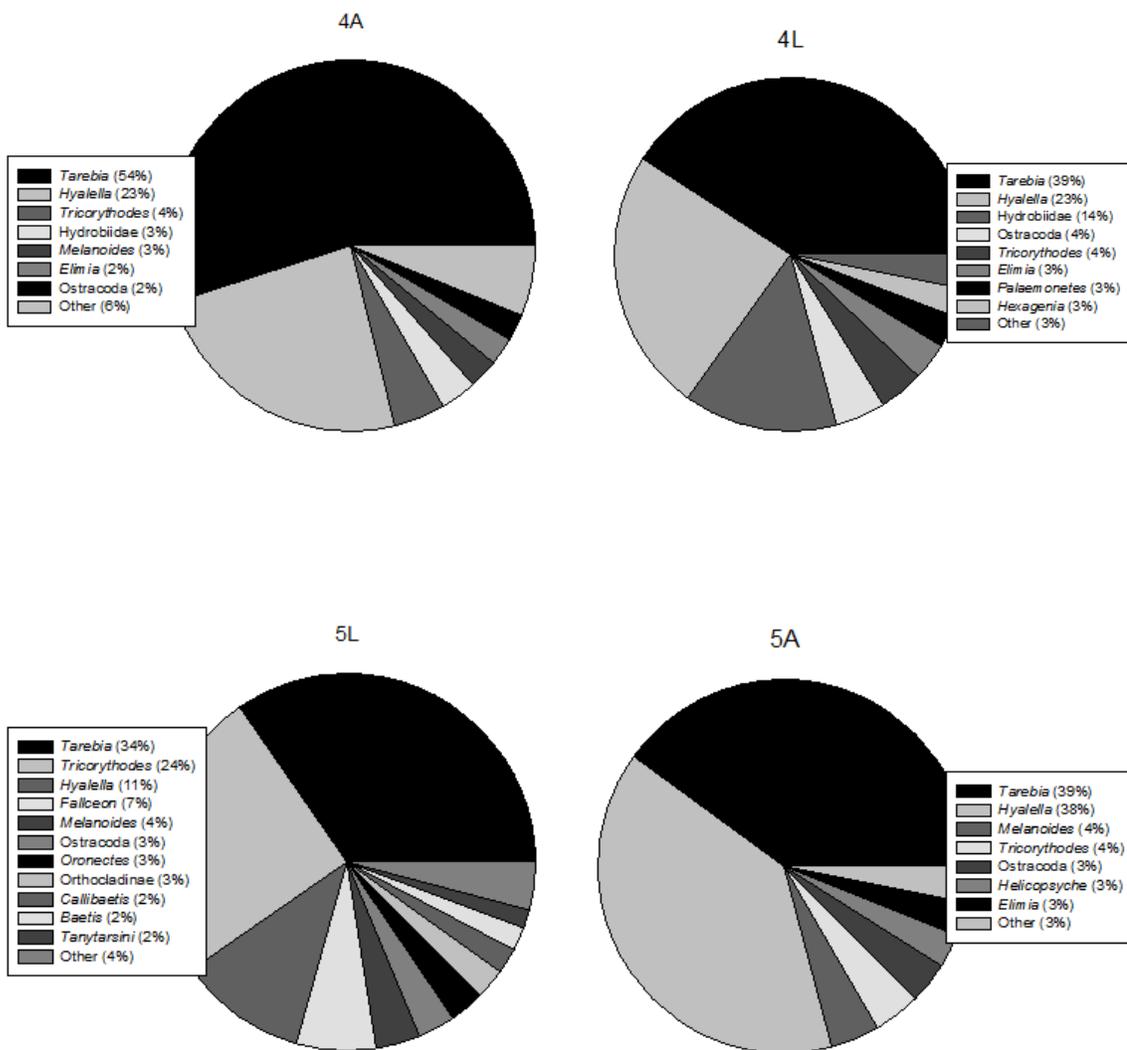
A - 3 (cont) Percent composition of fish captured by site throughout duration of study period.



A - 4. Percent composition of macroinvertebrates captured by site throughout duration of study period.



A - 4 (cont). Percent composition of macroinvertebrates captured by site throughout duration of study period.



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VITA

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